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Effects of β-glucan-starch interaction and extrusion cooking on the resistance of barley starches towards hydrolysis by amylases

by

Abdul Kipruto Faraj ©

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Dedicated to my mum, wife and children, brothers
and sisters for their love, patience and
encouragement.

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LIST OF ABBREVIATIONS

AACC	American association of cereal chemists
AOAC	Association of official analytical chemists
BAA	*Bacillus amyloliquefaciens* α-amylase
CAIT	Centre for agri-industrial technology
CDC	Crop development center
CL	Chain length
DP	Degree of polymerization
DSC	Differential scanning calorimetry
ES	Enzyme substrate complex
ES_{pr}	Productive enzyme substrate complex
ES_{np}	Non-productive enzyme substrate complex
EURESTA	European flair concerted action on resistant starch
FAO	Food and agricultural organization of the United Nations
FDA	United States Food and Drug Administration
FFAs	Free fatty acids
FT/IR	Fourier transform/infrared spectroscopy
GLM	General linear model
GOPOD	Glucose peroxidase assay
GS	Gelatinized starch
HAMS	High amylose maize starch
HB	Hull-less barley
H-BG	Hydrolysed beta-glucan

HDL	High density lipoprotein
HPAEC	High performance anion-exchange chromatography
IDF	Insoluble dietary fiber
Da	Daltons
LDL	Low density lipoprotein
LPLs	Lysophosholipids
mPa s	MilliPascal seconds
MW	Molecular weight
NMR	Nuclear magnetic resonance
PAA	Porcine pancreatic α-amylase
Pa s	Pascals seconds
RS	Resistant starch
SAXS	Small-angle X-ray scattering
SCFAs	Short chain fatty acids
SDF	Soluble dietary fiber
SEM	Scanning electron microscopy
SSEs	Single screw extruders
T_c	Conclusion temperature
TDF	Total dietary fiber
TEM	Transmission electron microscopy
T_m	Melting temperature
T_p	Gelatinization/peak temperature
TSEs	Twin-screw extruders

Chapter 1

INTRODUCTION AND OBJECTIVES OF THESIS

1.1 INTRODUCTION

Consumption of barley as human food has been low in many countries especially in the Western countries where most of this crop is grown. However, research findings on the health promoting properties of mixed-linkage β-(1→3)(1→4)-D-glucan (hereafter referred to as β-glucan) from mainly barley and oat, have generated considerable interest in barley (Anderson et al., 1984; Newman and Newman, 1991; Wood et al., 1994; Yokoyama et al., 1997; Bourdon et al., 1999; Hallfrisch and Behall, 2000; Keagy et al., 2001; Mälkki and Virtanen, 2001). These health benefits include lowering of blood cholesterol, attenuation of post-prandial serum glucose and insulin levels, immunostimulation and cancer fighting properties. In 1997, the United States Food and Drug Administration (FDA, 1997) approved a health claim ["soluble fiber from oatmeal, as part of a low saturated fat, low cholesterol diet may reduce the risk of heart diseases"] for products rich in oats β-glucan. This approval has boosted the use of oat as well as barley β-glucan containing products in human foods. The process of obtaining a similar health claim for products containing barley β-glucan is now in progress (Vasanthan, 2004). The development of new hull-less barley varieties (CDC-Candle, CDC-Alamo, Prowasonupana) with a high β-glucan content, has resulted in the promotion of barley as an excellent source of β-glucan.

Both *in vitro* and *in vivo* studies have confirmed the health promoting attributes of β-glucans in animals (Newman et al., 1989; Newman and Newman, 1991; Bhatty, 1999; Malkki and Virtanen, 2001). Human intervention studies have also confirmed some of these attributes, however, the actual mechanisms by which β-glucans lower cholesterol levels, attenuate serum glucose and insulin levels have not been fully elucidated, but is postulated to be related to the high viscosity and solubility of β-glucans in aqueous solutions (Kahlon et al., 1993; Yokohama et al., 1997). A better understanding of the behavior of β-glucan under different processing conditions and interaction with other food components is important for increasing commercial applications of barley flour and its isolated components, especially starch and β-glucan. The effects of β-glucan on the hydrolysis of starch and therefore its attenuation of post-prandial serum glucose level may promote the development of novel food products for certain groups of people such as athletes, diabetics, etc.

In recent years, considerable interest has been generated in resistant starch. Resistant starch (RS) has been defined by the European Flair Concerted Action on Resistant Starch (EURESTA) as the starch or products of starch degradation that escapes digestion in the small intestine of healthy individuals and may be completely or partially fermented in the colon (Englyst et al., 1992). It has been reported (Bjorck et al., 1986; Raben et al., 1994; Muir et al., 1995; Phillips et al., 1995) that resistant starch has both physiological and health benefits in humans. Resistant starch has been shown to have some health promoting attributes such as modulation of glucose released during digestion, cholesterol lowering properties, and prebiotic activity (native plant components that stimulate growth and/or activity of certain beneficial bacteria in the hind human gut). A number of researchers have shown that formation of RS is enhanced when high amylose starch is used as a starting material (Jane and Robyt, 1984; Sievert and Pomeranz, 1989; Eerlingen and Delcour, 1995; Thompson, 2000). High-amylose hull-less barley has been developed (Bhatty, 1999) and hence the processing of barley flour into food products containing a high content of RS may increase the demand of these products and therefore that of barley flour.

Obesity, and may be the preliminary steps in the progression of type 2 diabetes mellitus, are associated with the inability of the body to maintain a normal glucose level or requiring an excessive level of insulin to do so (Hallfrisch and Behall, 2000). Obesity is a health concern in North America and many developed nations. It has been proposed in recent years that low-carbohydrate diets are one of the options for easier weight loss (Ranum, 2004). Whether these diets work or not is still questionable, however, the popularity of low carbohydrate diets is growing (Ranum, 2004). One possibility of reducing starch in grain-based foods is to convert native starch to resistant starch, which might be more acceptable in the low-carbohydrate diets.

Extrusion cooking is an important and popular food processing technique. It has been used for processing breakfast cereals, pasta products, dextrinized flour, etc. Some of the advantages that have been attributed to this technique include low cost, high productivity, versatility and unique product shapes. Depending on the intended final product, various temperatures, moisture, shear and screw speed combinations can be used. Extrusion cooking of starchy grain flour (i.e corn, wheat, barley and oat flours) causes gelatinization of starch, an important event among other physico-chemical and functionality transformations. A number of investigators (Berry, 1986; Siervert and Pomeranz, 1989; Eerlingen et al. 1993a) have demonstrated that retrogradation of gelatinized starch induces the formation of resistant starch and thus, the need exists to investigate the effect of extrusion processing on some of the valuable nutrient components of barley, especially the hull-less barley varieties that are used mainly for human food.

1.2 OBJECTIVES OF THESIS

The objectives were to:

1. Investigate the effect of low purity barley β-glucan addition at different net β-glucan concentration levels on the kinetics of barley starch hydrolysis by porcine pancreatic α-amylase. It is expected that a better understanding of the physical and chemical properties of barley β-glucan and its interactions with other food components such as starch and its enzymic hydrolysates would promote its use in novel food products. This study may also provide an insight into the mechanisms by which barley β-glucan lowers serum glycemic response and modulation of insulin response.

2. Investigate the effect of extrusion cooking of pearled barley flour on its constituents, specifically starch, resistant starch and β-glucans. This is important as most of the studies on resistant starch formation have been performed on pure starch systems and only a few studies have involved complex systems such as pearled barley flour.

The proposed investigations will improve our understanding of the formation of resistant starch in barley flour and the effect of β-glucan on starch digestion. This could lead to the development of novel functional foods that can modulate the post-prandial blood glucose and insulin concentrations.

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Chapter 2

LITERATURE REVIEW

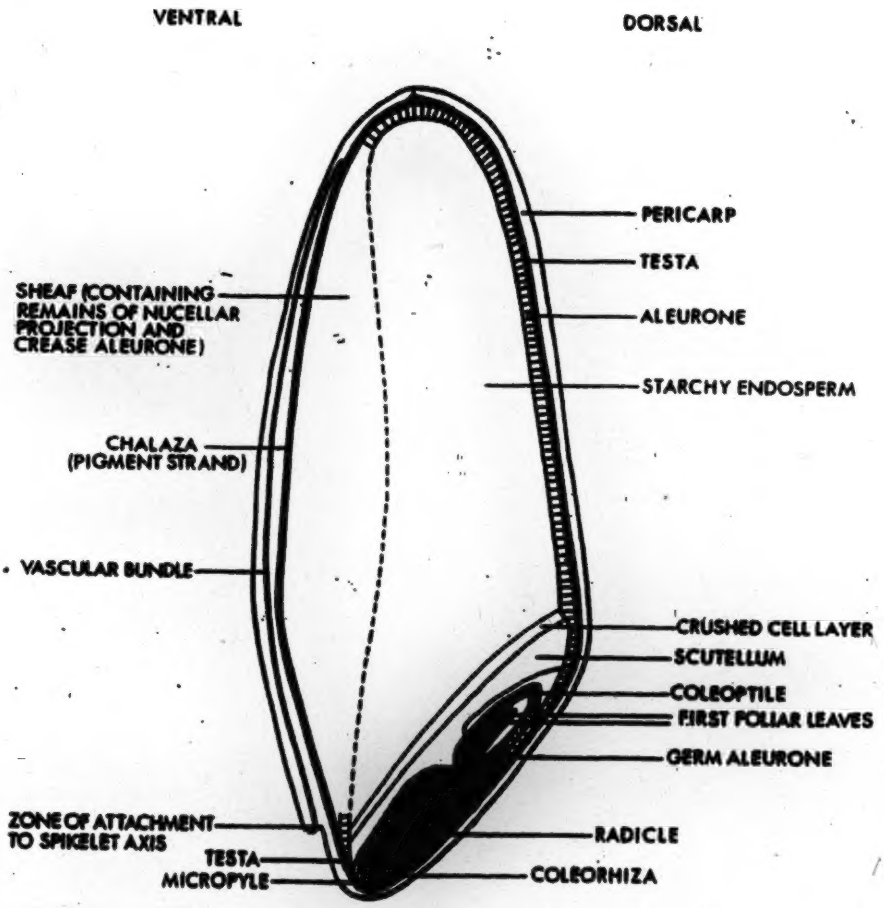
2.1 BARLEY GRAIN

2.1.1 Introduction

Barley, *Hordeum vulgare L.*, belongs to the tribe *Triticeae* and the genus *Hordeum* of the grass family Poaceae (Nilan and Ullrich, 1993). Barley, one of the oldest cereals in recorded history, was domesticated about 17,000 years ago along the River Nile Valley in Egypt (Nilan and Ullrich, 1993). Barley is a versatile crop and can be grown over a wide range of environmental conditions. Cultivated varieties are six–row and two-row type, depending on the number of fertile spikelets on the rachis. Two-rowed barley contains uniform, large and plump kernels that have lower husk content while the six-rowed barley are long and lean. The two-rowed barley is mostly used for malting/brewing and food processing, while the six-row type has been utilized mainly as livestock feed (Bhatty, 1993). Barley grains have traditionally been covered with hulls or husks, where the lemma and palea adhere to the caryopsis and do not thresh freely. However, hull-less barley (HB), also referred to as naked barley, has been developed in recent times, mainly for food and industrial applications (Bhatty, 1999).

2.1.2 Structure and Composition

Barley grain consists of one-seed fruit termed caryopsis (Fig 2.1). The hull or husk is the outer tissue of the caryopsis and constitutes 10-13% of the dry weight of barley grain. The hull is formed from the lemma and palea. In hulled barley, this is tightly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STARCHY ENDOSPERM
SHEAF (CONTAINING REMAINS OF NUCELLAR PROJECTION AND CREASE ALEURONE)
CHALAZA (PIGMENT STRAND)
VASCULAR BUNDLE
CRUSHED CELL LAYER
SCUTELLUM
COLEOPTILE
FIRST FOLIAR LEAVES
GERM ALEURONE
ZONE OF ATTACHMENT TO SPIKELET AXIS
TESTA
MICROPYLE
RADICLE
COLEORHIZA

Figure 2.1. Longitudinal section of a mature barley grain. (adapted with permission from Duffus and Cochrane, 1993).

attached to the caryopsis while in HB, the hull is loosely attached and falls off during harvesting and threshing. The caryopsis consists of the pericarp, seed coat, aleurone layer, germ and endosperm. The pericarp is about 2% of the total kernel weight and is mainly composed of cellulose, arabinoxylans and lignin. The seed coat or testa is 1-3% of the total kernel weight and is composed of cellulose, waxes and pigments with the anthocyanins being the predominant pigments (MacGregor, 1998). The aleurone layer comprising of arabinoxylans (60%), β-glucan (22%) and proteins (16%) contribute to about 5-10% of the total kernel weight. The embryo (2-4% kernel weight) is rich in lipids (13-17%), protein and amino acids (34%), sucrose and raffinose (5-10%), arabinoxylan, cellulose and pectin (8-10%) and ash (2-10%) (MacGregor, 1998). The endosperm, which contributes to 75-80% of the total kernel weight, is rich in starch and proteins and some lipids. The cell walls of the endosperm are composed of β-glucan (70-75%), arabinoxylans (20-25%) and proteins (5-6%). Other minor components in the barley grain are tocols (tocopherols and tocotrienols), minerals and vitamins.

A summary of barley grain composition is shown in Table 2.1. Hull-less barley generally contains more protein, starch, total dietary fiber and β-glucan compared to hulled barley (Table 2.2), however, it has lower grain yield per acre (Bhatty, 1999). The quality of the barley grain and its chemical composition is influenced by both growing conditions and genetic (varietal) factors. Barley is uniquely suitable for use in many feed and food products because of the availability of cultivars that have thin or no hulls, and normal, waxy or high amylose starch, low or high lysine, low or high β-glucan, and no known antinutritional factors [other than β-glucan] (Bhatty, 1993).

Table 2.1 Chemical composition of barley grain[1]

Component	Dry Weight (%, w/w)
Carbohydrates	78-83
Starch	63-65
Sucrose	1-2
Other sugars	1
Water-soluble polysaccharides	1-1.5
Alkali-soluble polysaccharides	8-10
Cellulose	4-5
Lipids	2-3
Proteins	10-12
Albumins and globulins	3-5
Hordeins	3-4
Glutelins	3-4
Nucleic acids	0.2-0.3
Minerals	2
Others	5-6

[1] Adapted with permission from MacGregor and Fincher (1993).

Table 2.2. Chemical composition of hulled and hull-less barley grains[1]

	Hulled (%, db)			Hull-less (%, db)			
Starch	52.1-63.8	49.4-63.1	53.7	23.9-64.4	49.4-66.2	60.1-73.8	59.7
Protein	8.7-10.5	9.3-15.5	15.9	11.3-18.1	10.6-21.9	12.8-17.8	16.5
Dietary fiber	18.9-23.8	18.1-27.5	18.6	13.5-34.5	13.6-20.2	11.0-16.6	13.8
β-Glucan	2.8-6.9	3.8-6.3	5.2	4.6-14.9	4.7-7.9	4.1-8.0	5.6
Lipid	2.2-3.5	2.1-3.1	-	2.9-6.2	2.1-3.7	-	-
Ash	2.3-2.6	1.9-2.3	2.8	2.0-2.3	1.3-2.1	1.8-2.2	2.1
Cellulose	3.5-4.7	3.1-7.0	4.1	1.4-4.1	1.7-5.0	-	2.0
Arabinoxylans	7.5-9.0	0.4-0.7[a]	6.5	4.8-12.2	0.7-0.9[a]	-	4.5
Lignin	1.4-1.7	1.0-1.9	2.0	0.7-1.1	0.5-0.9	-	9.0
Uronic acids	4.4-5.2	0.5-1.1[a]	-	3.4-5.7	0.5-0.7[a]	-	-
LMWC[b]	-	0.8-1.4	1.4	-	0.9-2.2	-	1.6
Reference	Andersson et al., 1999	Oscarsson et al., 1996	Xue et al., 1997	Andersson et al., 1999	Oscarsson et al., 1996	Bhatty and Rossnagel, 1998	Xue et al., 1997

[1] Source (Li, 2004) with permission.

[a] Water soluble fraction.

[b] LMWC, low molecular weight carbohydrates including glucose, fructose, sucrose, maltose, raffinose, and fructans.

2.1.3 Production and utilization

Barley is the fourth major cereal crop grown worldwide after wheat, rice and maize. World annual barley production for 2003 was 139.4 million metric tonnes (FAO, 2004). Canada is one of the largest producers of barley in the world with an annual average production of 13.5 million Mt (Agriculture Agri-food Canada, 2003). However, although the total barley acreage has generally increased in the last five years, the total barley production has decreased from 12.71 million Mt in 1998-99 to 7.28 million Mt in 2002-03 (Alberta Agriculture, Food and Rural Dev., 2004) as shown in Table 2.3. This is mainly due to the drought that was experienced in the last two years in Western Canada where most of the barley is grown. Most of the barley in Canada is produced in the Prairies provinces of Alberta, Saskatchewan and Manitoba.

Utilization of barley in Canada is mainly in the industries of feed (75%), malting and brewing (20%) and the remaining (5-6%) has been for other human food uses (Alberta Agriculture, Food and Rural Dev., 2004). On a global basis, 50, 30, 10 and 10% are utilized as feed, malt, food and whole seeds (for replanting), respectively. In Canada, it is predicted that the amount utilized as feed will be low for a short term while the beef industry recovers after the discovery of mad-cow disease in 2003. Previously barley cultivars were mainly being developed for either malting/brewing or feed purposes. However, new barley varieties (normal, waxy and high amylose) are now being developed for food and non-food uses.

In Western countries including Canada, a small fraction of food barley is used for breakfast cereals, baby foods, soups, stews, porridge, bakery blends and barley products (malt flour, pot and pearled barley flakes and grits). In West Asia and North Africa barley is consumed as pearled grain in soups, flour in flat bread and ground grain in cooked porridge (Bhatty, 1993). A substantial amount of barley is utilized in the human food chain in Japan and Korea. Barley is the second most important crop after rice in Korea and is used in several ways. These include pearled barley as a rice extender, *Aspergillus sp* inoculated pearled barley for soy paste and soy sauce production, roasted barley as tea or coffee substitute and barley-wheat composite flour for making cookies, cakes and noodles (Bhatty, 1993).

There is an increasing research interest in the utilization of barley as human food and industrial applications. This has been boosted by the recent recognition by the FDA (1997) of the health claim for oat β-glucan which can benefit human cardiovascular health. Although the claim is specifically for oats, it is known that barley and oats are the two cereal crops with high amounts of β-glucan. Research is now mainly focused on pearling and milling of barley grain, incorporation of barley flour and malt into baked and pasta products, extraction and characterization of the grain components, especially β-glucan, starch, protein, lipids, and tocols (Lazaridou et al., 2004; Storsley et al., 2003; Keagy et al., 2001; Burkus and Temelli, 1998, 1999; Bhatty, 1999; Izydorczyk et al., 1998a, b; Burkus, 1996; Newman and Newman, 1991). For profitability and sustainability of the barley industry especially in Canada (a leading producer of barley), novel ways of utilizing barley have to be developed in addition to the traditional usage in brewing and feed. A need exists therefore, for the incorporation of more barley in human diets as a nutritive and high fiber ingredient.

Table 2.3. Canadian barley production and disposition for period 1998-2003[1].

	1998-99	1999-00	2000-2001	2001-2002	2002-2003	**5 yr Aver[2]**
Area			Acreage (Million acres)			
Planted	11.447	10.895	12.556	11.614	12.719	**11.846**
Harvested	10.557	10.055	10.996	10.254	8.073	**9.987**
Item			Amount (Million metric tonnes)			
Total production	12.710	13.200	13.170	10.850	7.280	**11.440**
Feed and dockage	10.040	9.890	10.120	8.970	6.500	**9.110**
Human food	0.010	0.008	0.008	0.007	0.100	**0.009**
Industrial use	0.352	0.385	0.350	0.300	0.300	**0.337**
Seed requirements	0.380	0.437	0.406	0.444	0.420	**0.417**
Export grain	1.100	1.727	1.941	1.091	0.200	**1.212**
Export products	0.598	0.665	0.700	0.666	0.350	**0.596**

[1] Reference (Alberta Agriculture, Food and Rural Development, 2004).

[2] Five years average, 1998-2003. The total do not add up, because some items have been omitted.

Value-added processing in terms of fractionation of valuable components to be used in foods, pharmaceuticals, cosmetics and biotechnology will help diversify barley utilization. Interest in cereal β-glucan is fast growing due to its nutritional benefits, therefore understanding its interaction with food components (starch, proteins and lipids) and the implications of those interactions on sensory and nutritional properties of food warrants further research.

2.2 STARCH

2.2.1 Production and utilization

The United States of America (USA) and the European Union (EU) are the two largest producers and consumers of starch and starch products. The USA produces almost 51% of the total world starch production. In the EU starch is produced from maize, wheat and potatoes while in the USA it is almost entirely produced from maize. In Asia starch is commercially produced from rice, sorghum and sweet potatoes. The total world production of starch in 2000 was 48.5 million metric tones (LMC International, 2002). This was produced from maize (80%), wheat (8%), potatoes (5%) and cassava (5%).

Native starches have diverse properties and have been utilized for various food and industrial applications. Physical and chemical modifications have been employed to improve the properties of native starches and therefore widen the scope of its applications. The various starch derivatives are shown in Fig 2.2. However, there has been some concern in recent years in the use of chemicals to modify functional properties of native starch and their impact on human physiology (Vasanthan, 2004b). Currently, the trend is shifting towards the use of plant breeding techniques to improve functional properties of starch from various plant sources. This has resulted in the production of waxy (< 5% amylose content) and high amylose (> 35% amylose) maize, rice and barley cultivars. Therefore the need exists to use conventional or modified food processing procedures such as extrusion, to convert this raw food ingredient into high quality value-added human food with better sensory attributes.

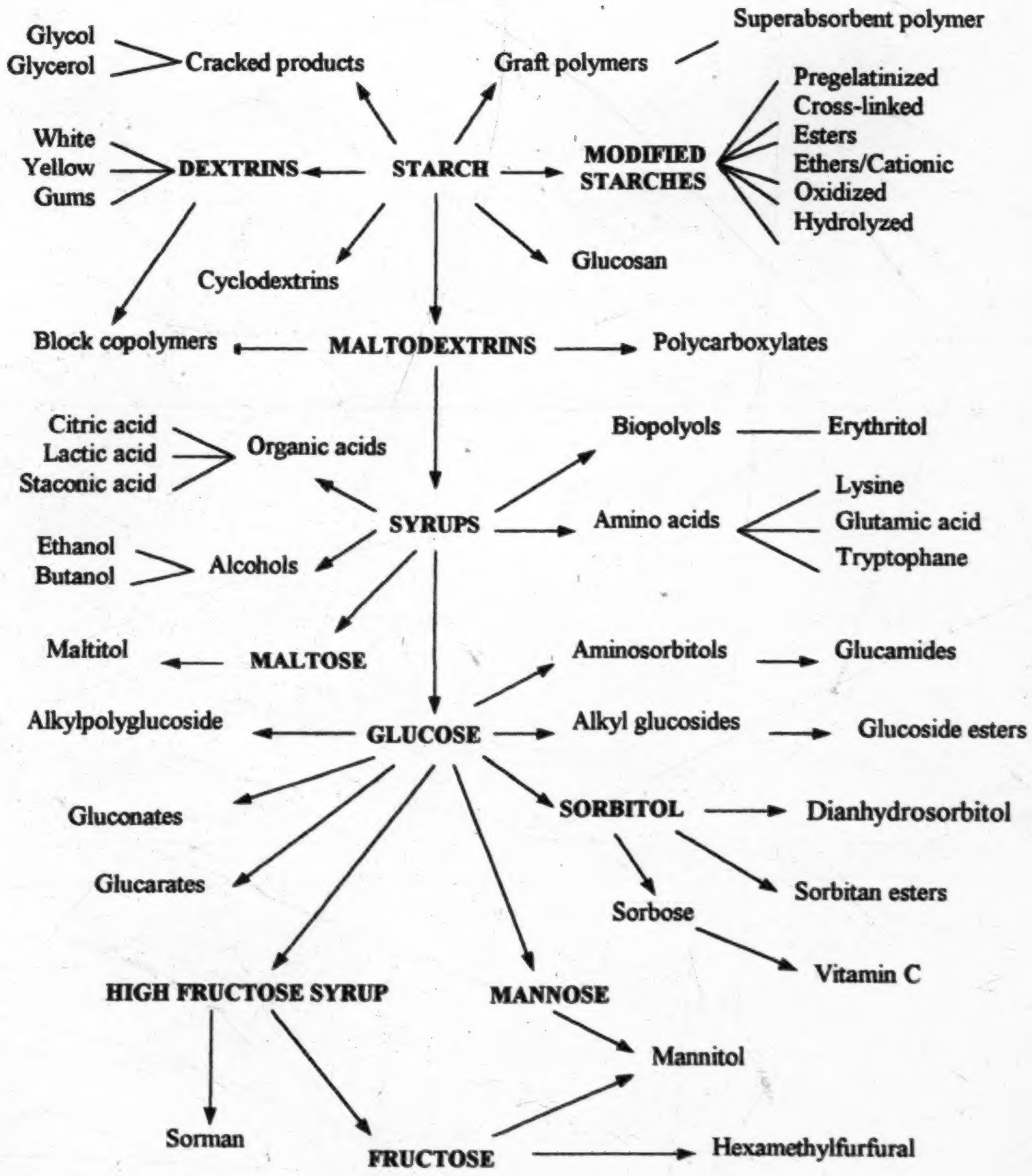
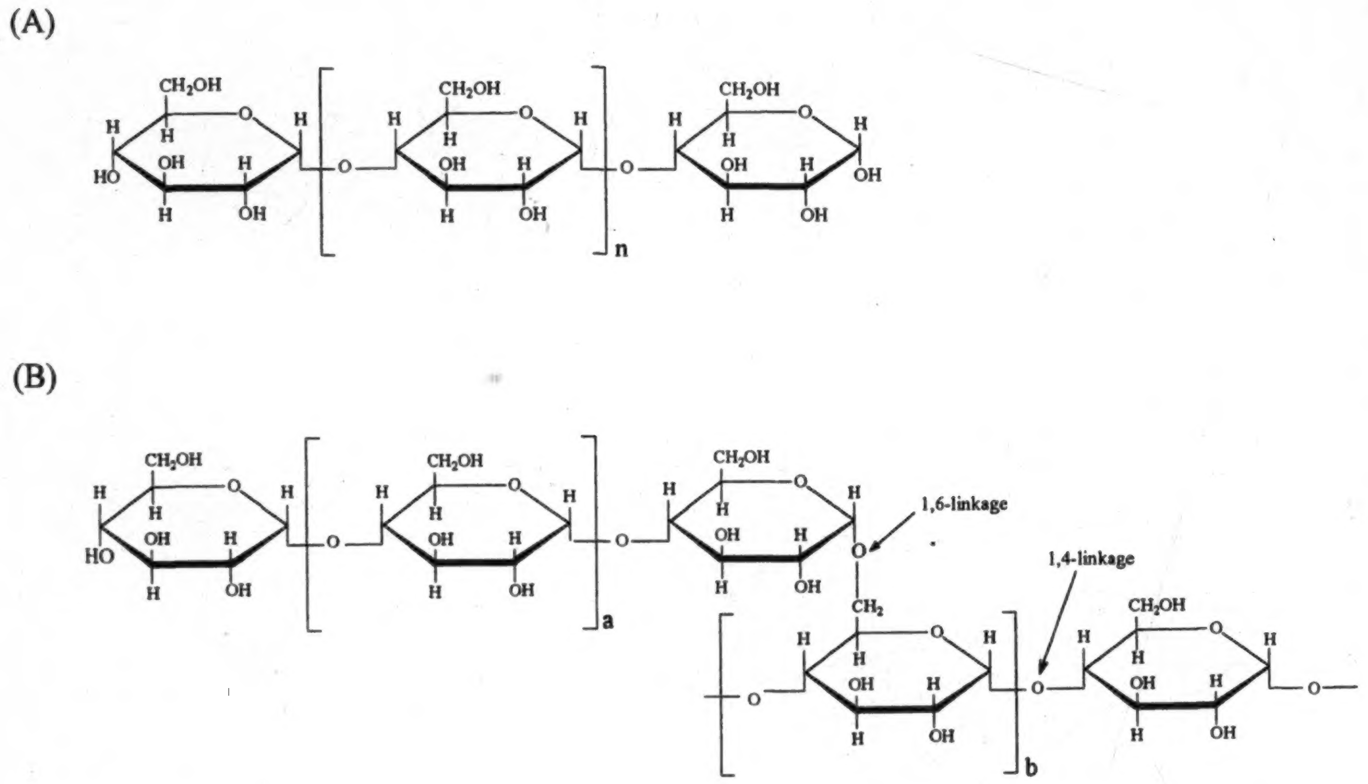

Glycol
Glycerol
Cracked products
Graft polymers
Superabsorbent polymer
White
Yellow
Gums
DEXTRINS
STARCH
MODIFIED STARCHES
Pregelatinized
Cross-linked
Esters
Ethers/Cationic
Oxidized
Hydrolyzed
Cyclodextrins
Glucosan
Block copolymers
MALTODEXTRINS
Polycarboxylates
Citric acid
Lactic acid
Staconic acid
Organic acids
Biopolyols
Erythritol
SYRUPS
Amino acids
Lysine
Glutamic acid
Tryptophane
Ethanol
Butanol
Alcohols
Maltitol
MALTOSE
Aminosorbitols
Glucamides
Alkylpolyglucoside
GLUCOSE
Alkyl glucosides
Glucoside esters
Gluconates
SORBITOL
Dianhydrosorbitol
Glucarates
Sorbitan esters
Sorbose
Vitamin C
HIGH FRUCTOSE SYRUP
MANNOSE
Mannitol
Sorman
FRUCTOSE
Hexamethylfurfural

Figure 2.2. Products derived from starch (adapted with permission from Rõper, 2002).

2.2.2 Composition and structure

Starch generally contains 85-90% (w/w) polysaccharides, 10-15% (w/w) moisture and minor non-starch components such as proteins, lipids and minerals (Tester, 1997). Cereal starches contain integral lipids and may also be contaminated with surface lipids (Morrison, 1993, 1995). The integral lipids are in the form of lysophospholipids (LPLs) and free fatty acids (FFAs) and have been positively correlated with the amylose fraction (Tester et al., 2004), while the surface lipids comprises of triglycerides, glycolipids and free fatty acids derived from amyloplast membrane and non-starch sources (Morrison, 1988, 1995). Starches from *Triticeae* sp contain almost exclusively LPLs while other cereal starches are rich in FFAs (Tester et al., 2004). Purified starches contain <0.6% protein which occur on the surface and, regardless of origin, are embedded within the matrix of granules (Tester et al., 2004). Both the starch lipids and proteins have potential to moderate starch functionality (Appelqvist and Debet, 1997).

Small amounts (<0.4%) of minerals, mainly calcium, magnesium, potassium, phosphorous, and sodium, are found in starches. However, (except for phosphorous) it has been shown that the minerals are of little functional significance (Tester et al., 2004). The major portion (97-99%) of isolated starch is composed of amylose and amylopectin, Fig. 2.3. The ratio of these components depends on species and cultivars (Galliard and Bowler, 1987). The amount of amylose in normal starches constitutes about 15-30% of the total starch. Waxy starches from maize, barley, wheat, potato, sorghum and rice mutants have about 0-10% amylose. The amylose content in amylomaize and high-amylose barley starches is about 35-70% (Shannon and Garwood, 1984).



(A)
CH₂OH
H
OH
HO
OH
O
n
(B)
a
b
CH₂
1,6-linkage
1,4-linkage

Figure 2.3 The structure of (A) amylose: average chain length n = ca. 1000 and (B) amylopectin: average exterior chains a = ca. 12-23 and interior chains b = ca. 20-30 (modified from Tester et al., 2004).

2.2.2.1 Amylose

Amylose consists mainly of relatively long chains of α (1→4) linked D-glucose units as illustrated in Fig. 2.3 (a). It exhibits a wide distribution of molecular sizes, 10^4-10^6 (Gidley, 2001) and has an average degree of polymerization (DP) of 1,800 (MacGregor and Fincher, 1993). Cereals have much smaller average molecular size compared to root and tuber starches. Previously, amylose has been referred to as a linear molecule, but it has been shown to have a low level of branching (Manners, 1985; Hizukuri, 1996). The extent of branching depends on the origin of the amylose and it increases with molecular size of amylose from a particular source (Takeda et al., 1987; Yoshimoto, 2000). The presence of more branching results in decreased susceptibility to β-amylolysis. The side chains of amylose have not been characterized, but generally, amylose molecules contain 0.27-0.68% branching points with an average chain length of 100-550 anhydrous glucose units (Takeda et al., 1987; Hizukuri et al., 1997). Amylose can be completely hydrolysed to maltose by the combined action of β-amylase and debranching enzymes such as pullulanase or isoamylase.

The conformation of amylose in aqueous solution has generated a lot of controversy and many models have been proposed. Hollo et al. (1961) proposed a tightly wound helix. They suggested that the helical segments are interspaced by regions of random coils while Banks and Greenwoods (1971) postulated that amylose in neutral aqueous solutions exist in the form of random coil with no helical segments. However, it is widely accepted that in neutral aqueous solution, amylose tends to be slightly helical due to the natural twist present in the α- and β-conformation of the glucose units. It behaves as a random, flexible coil which consists of extended helical segments stabilized by intermolecular hydrogen bonds, interspaced with non-helical segments (Appelqvist and Debet, 1997). Its typical hydrodynamic radius in solution is 7-22 nm^2 (Buleon et al., 1998b). Hydrodynamic radius is a measure of the volume occupied by a polymer in solution depending on the molecular weight. The concept of double helixes (Fig 2.4) is widely accepted since it explains that in the absence of a complexing agent, amylose helixes are best stabilized by an interaction between the two strands (Appelqvist and Debet, 1997). The two chains fit together compactly with the hydrophobic parts of the glucose units in close contact inside the structure, and the hydroxyl groups on the outside of the double helix in a position suitable for hydrogen-bonding between the chains in water (Appelqvist and Debet, 1997).

It has been observed that due to its chain length, the linear portion of amylose can form complexes with various ligands, e.g; lipids, aliphatic alcohols (Morrison, et al., 1993a, 1993b) and such complexes result in a V-polymorph pattern (Fig 2.5). Morrison et al. (1993c) reported that all the lipids in native starches are complexed with some of the amylose, however, more recent data (Kiseleva et al., 2003) suggested that not all of the lipids are complexed.

2.2.2.2 Amylopectin

Amylopectin is the major component of most starches. It is composed of α-(1-4) linked glucose molecules interconnected through branches at α-(1-6) bonds as illustrated in Fig. 2.3 (b). The branching makes 4-5% of the inter chain α-(1-6) branches and restricts the extent of hydrolysis by β-amylase. The molecular weight of amylopectin is 10^6-10^8 Da, with hydrodynamic radius of 21-75 nm^2 (Buleon et al., 1998b) making it one

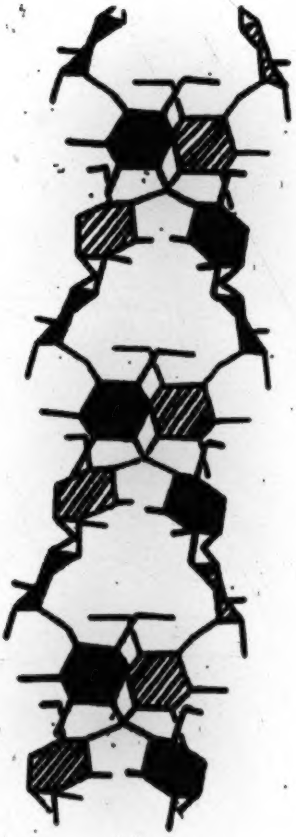


Figure 2.4 Double-helix structural model of amylose (adapted with permission from French and Murphy, 1977).

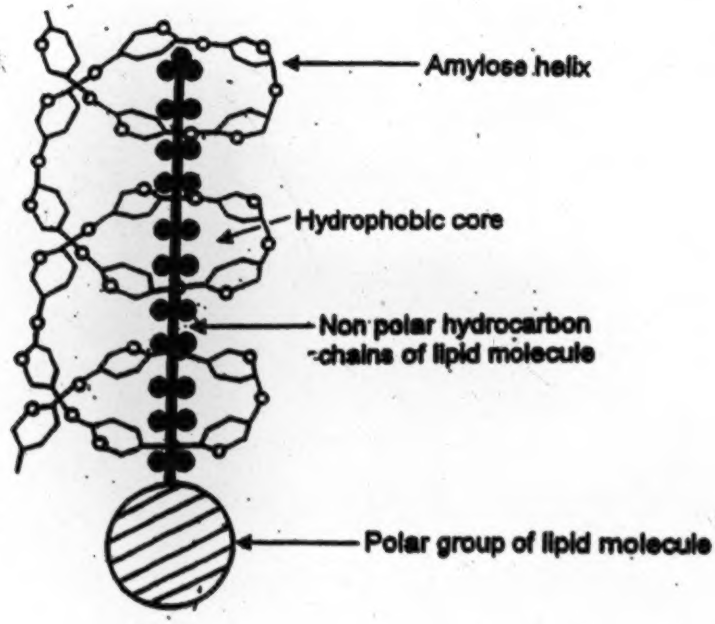

Amylose helix
Hydrophobic core
Non polar hydrocarbon chains of lipid molecule
Polar group of lipid molecule

Figure 2.5. Schematic representation of amylose-lipid complex (adapted with permission from Carlson et al., 1979).

of the largest occurring natural polymers. A consequence of branching is that for its molecular weight the amylopectin molecule is relatively compact (Parker and Ring, 2001). Amylopectin has a much wider molecular weight distribution compared to amylose but it does not form the characteristic dark blue color complex with polyiodide ions in aqueous solution. The average size of unit chains is 20-25 glucose units. These short chains restrict the formation of complexes, for example with lipids or iodide ions (MacGregor and Fincher, 1993). In high amylose starches, the amylopectin has a lower degree of branching and higher average chain length than those in waxy and normal starches (Tester et al., 2004).

Amylopectin contains a small amount of phosphate groups. These phosphate monoesters may play important roles in starch such as enhanced paste clarity, high paste viscosity, low gelatinization temperature, significant shear thinning, and slow retrogradation rate (Kasemsuwan and Jane 1994). The amount of the phosphate monoester vary in different starches ranging from 200-1000 ppm in potato, 40-150 ppm in root and legume to less than 200 ppm in cereal starch and, 110-260 ppm in amylomaize (Hizukuri, 1996).

The molecular arrangement of amylopectin has been the subject of numerous studies. A number of structures have been proposed but the most widely accepted model at the moment is that derived from the cluster model of French (1972) and Robin et al., (1974). A refinement of this cluster model has been proposed by Hizukuri (1986) based on the polymodal distribution of the chain profile elucidated from gel permeation HPLC of debranched amylopectin (Fig. 2.6). It is proposed that amylopectin molecules contain three types of unit chains.

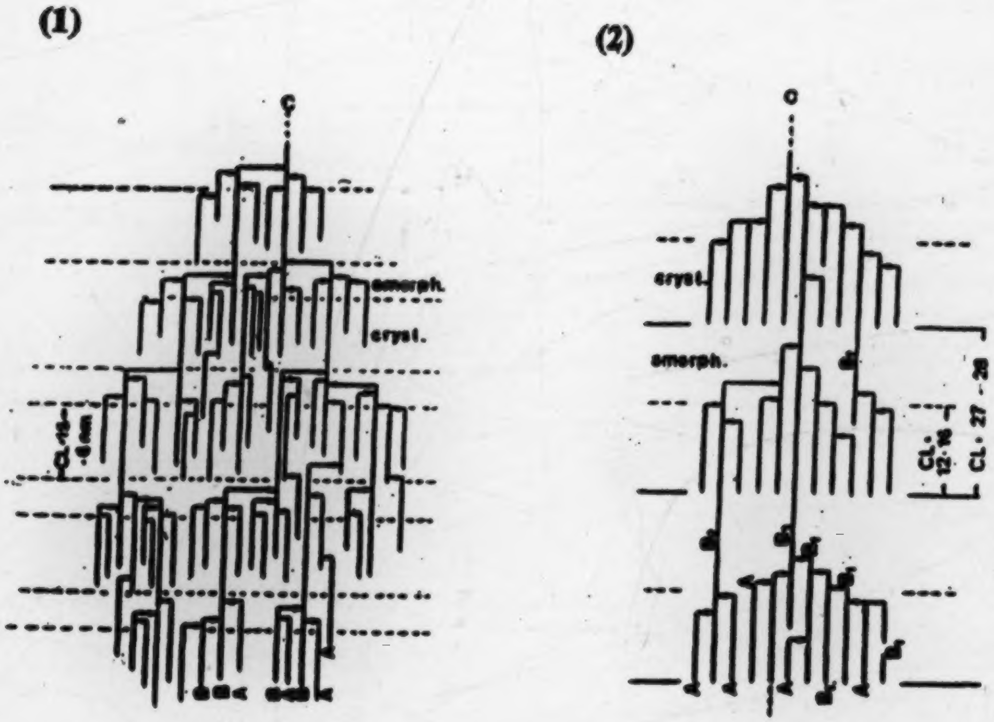

(1)
C
amorph.
cryst.
(2)
C
cryst.
amorph.
CL : 12-16
CL : 27 - 28

Figure 2.6. Amylopectin cluster models as proposed by (1) Robin et al., 1974 and (2) Hizukuri, 1986. A: outermost branches, B: inner branches and C: the chain that carries the only reducing group. Amorph.=amorphous region, Cryst.=crystalline region, CL=chain length (adapted with permission).

The short or A-chains are unbranched and linked to the molecule through their reducing end group by α-(1-6) glycosidic bonds. The long or B-chains are similarly joined to the molecule but carry one or more A-chains. There is only one C-chain, which carries the reducing group of the molecule. The unit chains are thought to concentrate in clusters, with the A and B_1-chains occurring within individual cluster. Hizukuri (1986) introduced the concept of B_1, B_2, B_3, etc, where B_2-chains join two clusters while B_3 join three clusters. The A and B_1 chains are the most exterior and form double helixes (and potentially crystallites) with native granules (Tester et al., 2004).

Debranching of amylopectin using isoamylase or pullulanase (enzymes which hydrolyse the α-(1-6) glycosidic bonds) followed by fractionation of the linear chains using chromatographic techniques revealed that debranched amylopectin has di, tri or polymodal distribution. By debranching amylopectin with pullulanase, MacGregor and Morgan (1984) showed a trimodal distribution of unit chains. The most abundant peaks were the shortest chains, DP 10-12 and may represent the A-chains. Another peak at about DP 18-20 may contain some A-chains but most likely represented the B_1 chains. The longest of the peaks at DP 40-50 may represent B_2 and B_3. Hizukuri (1986), using potato, tapioca, waxy rice and kuzu starches, showed a polymodal distribution where the debranched fractions A, B_1, B_2, B_3 and B_4 represented chain groups of amylopectin with an average chain length (CL) of 12-16, 20-24, 42-48, 69-75, and 101-119, respectively. The A- and B_1-chains accounted for 80-90% (mole basis) of the total chains which represented outer chains in a single molecule, B_2-chains 10%, B_3-chains 1-3% and B_4-chains 0.1-0.6%.

Branched chain length distribution of amylopectin is related to the crystalline polymorphs and its profile is a characteristic of each starch even in the same polymorph type (Li et al, 2003). The A-type starches have shorter peak DP and shorter average chain length with relatively higher proportion of shorter chains (DP 6-12) than the B-type starches. The C-type starches have intermediate chain length and amounts (Hizukuri, 1985, 1986; Jane et al., 1999). However, amylopectins from amylomaize have relatively longer chain length and higher proportion of long chains (DP>37) than waxy starches and normal maize starches (Jane et al., 1999).

A number of researchers have shown that branch chain length distribution of amylopectin influences gelatinization and retrogradation characteristics, pasting properties and susceptibility towards enzyme and acid hydrolysis (Shi and Seib, 1992, 1995; Cheetham and Tao, 1997; Fredriksson et al., 1998; McPherson and Jane, 1999; Jane et al., 1999; Gerard et al., 2001; Matveev et al., 2001).

2.2.3 Ultra structure of starch granules

It is now widely accepted that starch granules have crystalline and amorphous regions. The level of crystallinity in granular starch is 15-51%, with an average of about 35% (Tester et al., 2004). The origin of crystallinity is understood to be due to the intertwining of the outer chains of amylopectin in the form of double helices (Tester et al., 2004). These associate together to form ordered regions or crystalline lamellae. Adjacent double helices give rise to regular three-dimensional patterns. These lamellae structure (clusters) as depicted in Fig. 2.7, was originally proposed by French (1972) and is now widely accepted in principle.

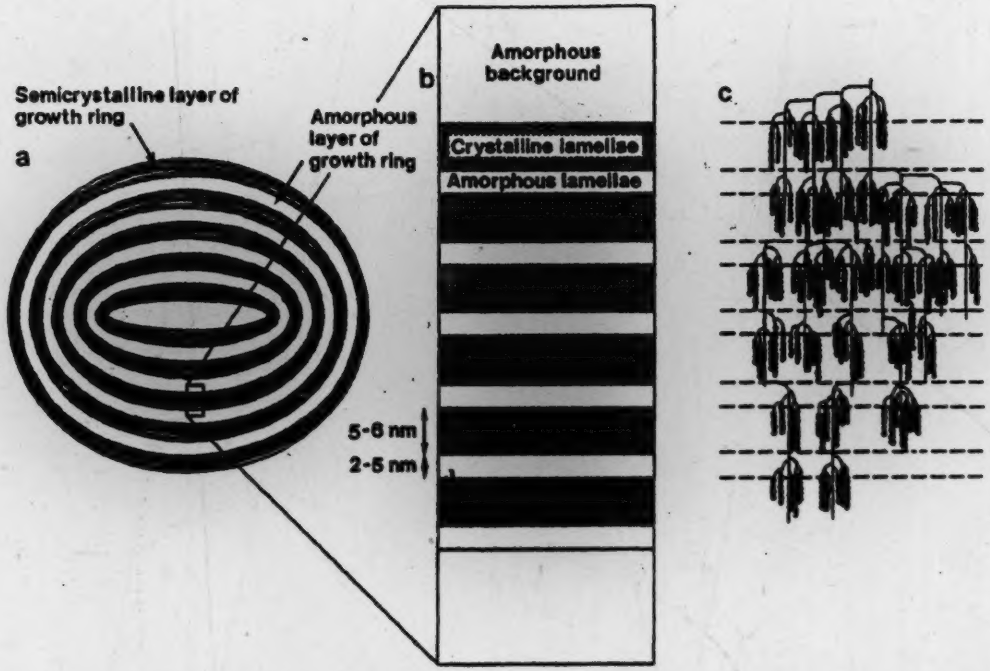

Semicrystalline layer of growth ring
a
Amorphous layer of growth ring
b
Amorphous background
Crystalline lamellae
Amorphous lamellae
5-6 nm
2-5 nm
c

Figure 2.7. Schematic diagram of starch granule structure. (a) a single granule with alternating amorphous and semicrystalline layers, representing the growth rings; (b) expanded view of the semicrystalline layer of a growth ring, consisting of alternating crystalline and amorphous lamellae; (c) the cluster structure of amylopectin within the semicrystalline layer of the growth ring. (adapted from Jenkins et al., 1994, with permission)

The growth rings (lamellae) represent periodical diurnal deposition of starch (Tester et al., 2004). The alternating crystalline and amorphous lamellae of amylopectin can only be observed in hydrated starch and not in dry starch (Cameron and Donald, 1992). The optimum moisture content for maximum crystallinity is observed at about 27%, while a minimum of 8% water is necessary for starch to give an X-ray diffraction pattern (Imberty and Perez, 1988). The amorphous region represents mainly the α-(1-6) branching points. A large portion of a starch granule is in the amorphous phase. The amorphous phase is interdependent but incompatible with the crystalline phase within granules. However, there is no sharp distinction between these two phases (French, 1984) and not much of the amorphous phase has been elucidated.

The crystalline pattern types within starch granules from various botanical sources are known and have well defined X-ray diffraction patterns that have been classified as A- B- and C-type polymorphs. They are designated as A-, B- and C-types based on their d-spacing calculated from their X-ray diffraction patterns (Imberty et al., 1991). Scanning electron microscopy (SEM) of fractured surfaces from amylase treated starches plus transmission electron microscopy (TEM) of chemically treated thin sections of starches have shown a radial periodicity or 'growth rings' (French, 1984). Crystallization of the A-type polymorph over the B-type was shown to be favored under conditions of shorter chain length values, higher temperature, high concentrations and the presence of salts and water soluble alcohols (Gidley, 1992). In addition, branch length of the amylopectin molecule (Hizukuri et al., 1983; Hizukuri, 1986; Gidley, 1987; Yuan et al., 1993) and water content (Imberty et al., 1991) are suspected to influence the type of packing.

According to Wu and Sarko (1978a, 1978b), both the A and B type amylose polymorph form a six fold, right-handed, parallel-stranded double helix and that the packing of the duplexes into the crystal lattice is antiparallel in both structures. The authors showed that the differences between the polymorphs are mainly due to the water content and the location of the water molecules (Fig 2.8). However, studies carried by Imberty and coworkers (Imberty et al., 1988, Imberty and Perez 1988) established that the A-type polymorph and the B-type are characterized by packing of left-handed parallel-stranded duplexes. Pfannemüller (1987) showed by studies on short amylose fibers of uniform length using X-ray diffraction analysis combined with computer modeling techniques, that the unit cell of the B-type amylose contains 36 water molecules loosely associated in a channel formed by the hexagonal packing of the helixes, while in the orthogonal unit cell of the A-type amylose, this channel was occupied by another double helix and 8 water molecules distributed in the intestitial spaces between the strands. He showed that the degree of crystallinity and the formation of A- and B-type amyloses are largely dependent on the chain length. In the A-type, there is a closely packed arrrangement of double-helixes, whereas in the B-type, the structure is more open with a greater amount of interhelical water (Appelqvist and Debet, 1997). Amylopectin of the B-type starches have longer chain length than those of the A-type (Hizukuri et al., 1983). Generally, the A-type pattern is exhibited by cereal starches and the B-type is shown mainly by tubers and high amylose starches.

The C-type pattern is an intermediate between the A- and B-types and is mainly observed in legumes. Initially there was some debate as to whether the C-type is a distinct polymorph or a mixture of A and B polymorphs with intermediate behavior and

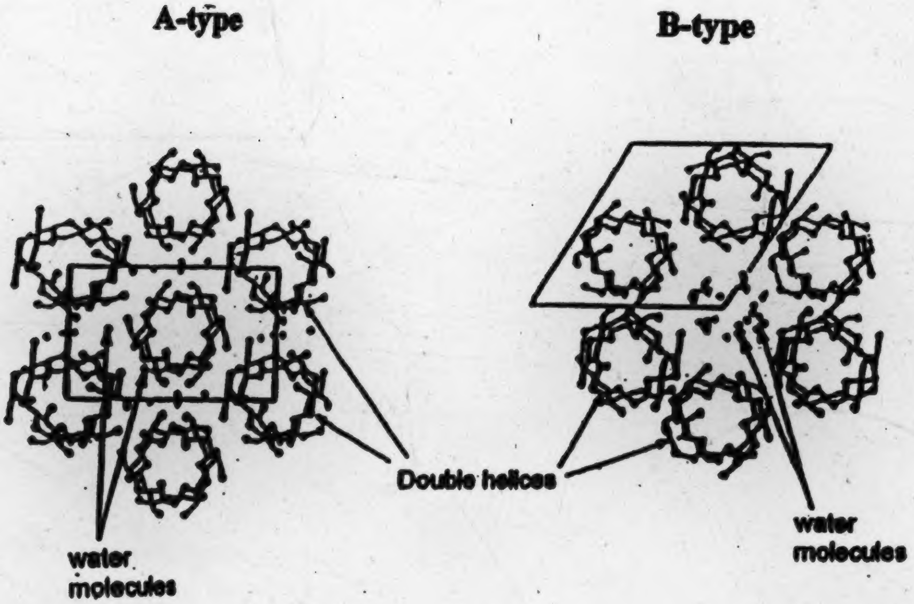

A-type
B-type
Double helices
water molecules
water molecules

Figure 2.8. Double helix packing arrangement of amylose crystallites in A- and B-polymorps (adapted with permission from Wu and Sarko, 1978a, b).

(Wu and Sarko, 1978a, b). Using pure malto-oligomers, Pfannermüller (1987) showed that the C-polymorph was a mixture of both the A- and B-polymorph. Further classification of the C type as Ca, Cb and Cc is on the basis of their resemblence to the A-type and B-type and between the two types, respectively (Hizukuri, 1986).

Amylopectin is the main crystalline component of the starch granule. This is supported by the observation that under appropriate conditions amylose may be leached out from the starch granules without seriously impairing granule crystallinity (Zobel, 1988) and waxy maize starch contains no or small amounts of amylose yet it is semi-crystalline. Amylose does not appear to have any significant effect on the crystallinity in normal and waxy starches which display strong birefringence (French, 1972; Banks and Greenwood, 1975; Blanshard, 1987; Zobel 1988; Hoover, 2001). However, in high amylose starches the amylose may contribute significantly to the crytallinity (Banks and Greenwood, 1975; Tester et al., 2004).

The exact location of amylose relative to amylopectin is not fully understood. It has been shown that amylose is located in bundles between amylopectin clusters (Blanshard, 1987; Zobel, 1992). However, Jane et al. (1992) and Kasemsuwan and Jane (1994) showed that amylose was interspersed as individual molecules both in the amorphous and crystalline regions of the granule rather than being in bundles. Gallant et al., (1997) have suggested that amylose in granules may occur in the amorphous granular rings, inter-crystalline amorphous lamellae, between crystallites within crystalline lamellae or in the radial channels and central cavities.

The precise structural role of the amylose in starch granule is not completely understood. Jenkins and Donald (1995) investigated the effect of varying amylose content on the internal structure of maize, barley and pea starches. The amylose content of these starches were: maize (waxymaize (0%), normal maize (28%), and amylomaize (70%); pea (low amylose pea (13%), wild-type pea (30%) and wrinkled pea starch (70%). The actual amylose contents of the barley starch used were not known, however, the varieties used, in order of increasing amylose contents were waxy Bowman, Bowman and high-amylose Glacier. The authors showed that increase in amylose content increases the crystalline lamellae size but reduces the electron density of small-angle X-ray scattering (SAXS) pattern, which suggest that the amylose acts to disrupt the packing of the amylopectin double helices within the crystalline lamellae. Jenkins and Donald (1995) proposed two possible mechanisms for this disruption. Mechanism 1 (Fig. 2.9) involves amylose co-crystallizing with the amylopectin, pulling the amylopectin chains out of register in the process. Mechanism 2 involves amylose chains oriented transverse to the lamellar stack, penetrating the amorphous lamellae and introducing disorder.

2.2.4 Effect of heating and cooling on starch

2.2.4.1 Gelatinization

In the presence of excess water and heat, starch granules undergo an order-disorder phase transition that has been termed gelatinization. The process involves granule hydration, swelling, loss of birefrigence, crystallites melting, uncoiling or dissociation of the double helices and the leaching of amylose and amylopectin chains (Appelqvist and Debet, 1997).

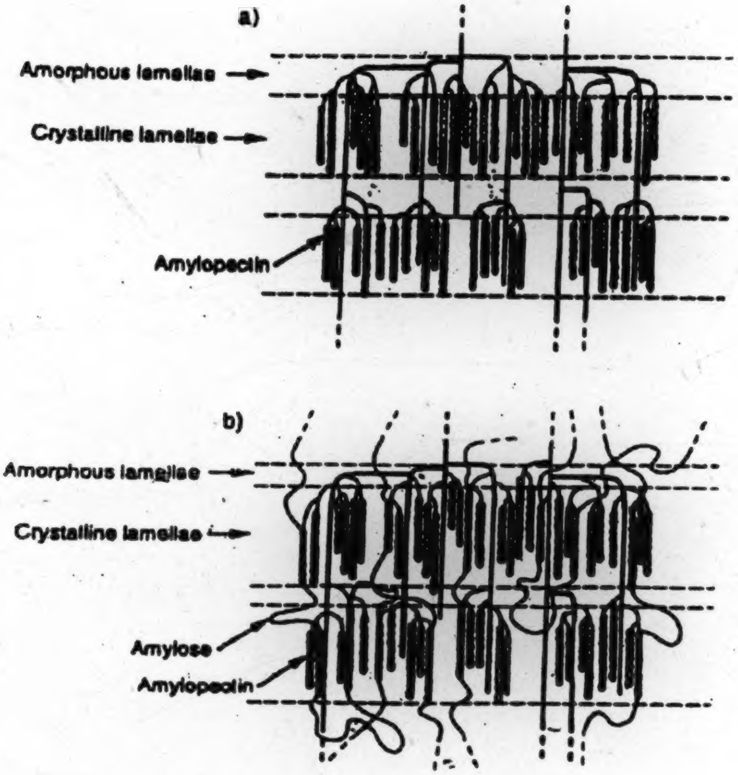

a)
Amorphous lamellae
Crystalline lamellae
Amylopectin
b)
Amorphous lamellae
Crystalline lamellae
Amylose
Amylopectin

Figure 2.9. One possible mechanism to explain the disruption of amylopectin double helical packing by amylose. (a) Amylopectin structure with no amylose present. Small crystalline lamellar present. (b) Co-crystallinity between amylose and amylopectin pulls a number of the amylopectin chains out of register. The crystalline lamellar size increases. (adapted with permission from Jenkins and Donald, 1995)

In the initial stages of gelatinization, the granules will start absorbing water and swell, and the molecular order of the granule structure disappears. This is manifested by loss of birefringence as observed under polarized light. The crystals then start to melt. Glass transition of the amorphous regions, at the glass transition temperature (T_g) is an important property of partially crystalline materials such as starch granules. The amorphous regions are transformed from a rigid glassy to a mobile rubbery phase when passing T_g during heating (Jacobs and Delcour, 1998). The softening of the amorphous regions is required before melting of crystallites can take place (Slade and Levine, 1987). Therefore, T_g always precedes gelatinization.

Many methods have been used to determine starch gelatinization such as viscoamylography, Kofler hot-stage microscopy (Watson, 1964), pulsed NMR (Lelievre and Mitchell, 1975), differential scanning calorimetry (DSC) (Donovan, 1979), enzymic digestibility (Shiotsuba, 1983), X-ray diffraction (Zobel et al., 1988), small angle X-ray scattering (Jenkins, 1994) and small angle neutron scattering (Jenkins, 1994). However, DSC is the most widely used method for determining starch gelatinization. DSC measures the gelatinization parameters [onset (T_o), peak (T_p), conclusion (T_c) temperatures and enthalpy (ΔH)] (Zobel, 1984; Tester and Debon, 2000; Hoover, 2001). The T_c–T_o represents the gelatinization range. It has been postulated that the molecular architecture of the crystalline regions, which corresponds to the distribution of amylopectin short chains (DP 6-11) influences the DSC parameters and not the proportion of crystalline region, which corresponds to the amylose to amylopectin ratio, (Noda et al., 1996). When starch granules are gelatinized their ultrastructure is disrupted and rendered fully accessible to hydrolytic enzymes. Gelatinization is of great importance to many food processing operations as many food processes depend on proper gelatinization of starch to produce the desired texture.

2.2.4.1.1 Factors influencing gelatinization characteristics

2.2.4.1.1.1 Water content

A number of explanations for the melting behavior of starch in excess or limited amount of water have been offered. Most of these explanations do not account for the fact that gelatinization of some starches starts at the hilum and proceeds to the periphery of the granule (Hoseney et al., 1986). In excess water gelatinization is a swelling driven process (Donovan, 1979; Jenkins and Donovan, 1998). Initially the amorphous regions swell and destabilize the amylopectin crystallites by ripping apart the edges of the crystallites (Appelqvist and Debet, 1997). A single endothermic peak in DSC is usually observed under conditions of excess water. This was initially thought to correspond to the melting of amylopectin crystallites (Tester and Morrison, 1990). However, Gidley and Cooke (1991) and Cooke and Gidley (1992) suggested that the observed DSC endotherm was mainly due to the melting of the double helices and that melting of the crystallites and double helices could be happening concurrently on heating. Their results suggested that the forces holding the granule together are mostly at the double helical level rather than the crystallite level and the gelatinization enthalphy (ΔH) may correspond to the disruption of the double helical order (Appelqvist and Debet, 1997). The disruption of individual crystallites occurs rapidly but over a limited temperature range (1-2 °C) for a single granule and a wide range (10-15 °C) for whole population of granules with a typical endothermic enthalphy values of 10-25 J/g (Biliaderis, 1983; French, 1984; Liu and Lelievre, 1993; Eliasson and Gudmundsson, 1996; Fredriksson et al., 1998).

Under conditions of intermediate or limited water, a biphasic endotherm occurs (Donovan, 1979; Burt and Russel, 1983; Hoseney et al., 1986). A number of suggestions and interpretations have been offered for the observed double endotherms. Russel (1987a,b) interpreted the observed double endotherms are due to double helices associated with short-range ordering involving amylose and amylopectin followed by melting of the crystallites, while Biliaderis (1990) suggested that it could be due to recrystallization. However, it has been suggested by Evans and Haisman (1982), Liu et al. (1991) and Liu and Lelievre (1992) that the endotherm peaks observed correspond to the order-disorder transition due to the range of crystallite stabilities occurring at different diluent levels. That is, the positions of the endothermic DSC traces are governed by the melting of the granules of different stabilities at different volume fraction of the diluent [water] (Appelqvist and Debet, 1997).

2.2.4.1.1.2 Amylose:amylopectin ratio

The relationship between amylose to amylopectin ratio and thermal properties of different starches have been extensively studied. Generally the waxy starches from wheat, barley, maize and potatoes show higher gelatinization temperatures than their normal counter parts (Gudmundsson and Eliasson, 1992; Jane et al., 1999; McPherson and Jane, 1999). Several researchers have postulated that the high gelatinization temperature and enthalphy may be related to the high degree of crystallinity caused by high amylopectin content (Cheetham and Tao, 1998; Matveev et al., 2001). It has also been shown that amylopectin chain length and therefore the crystalline type and the ratio of the A- and B-polymorphs influence thermal properties of starch (Cheetham and Tao, 1997; Gerard et al., 2001; Matveev et al., 2001). The longer the amylopectin chain length, the more crystalline the starch structure and therefore, the higher the gelatinization temperature. However, potato starch has a higher proportion of long branch chains than the cereals but a low gelatinization temperature. Kasemsumwan and Jane (1994) and Jane et al. (1999) attributed this to the high phosphate monoester derivatives present in potato starch. Muhrbeck et al., (1991) using potato starch showed that the higher the degree of phosphorylation the lower the gelatinization enthalpies. The authors suggested that this effect may be due to the phosphate monoesters hindering the build up of crystal structure of the starch. The phosphate monoesters promote its hydrophilic nature by wedging apart individual starch grains with negatively charged phosphate groups and therefore interfere with the crystallinity of the amylopectin (Lim et al., 1994)

2.2.4.1.1.3 Salts

Salts can influence the gelatinization temperature of starch. Depending on the nature and concentration of the salts they can either increase or decrease the gelatinization temperature (Gough and Pybus, 1973). When starch is heated in the presence of water it gelatinizes with an endothermic enthalpy, but in the presence of concentrated calcium chloride (>4M) it gelatinizes with an exothermic enthalpy (Evans and Haisman, 1982). Sodium sulphate substantially increases gelatinization temperature, while sodium thiocynate (>2M) gelatinizes starch at room temperature (Evans and Haisman, 1982). However, Gough and Pybus (1973) showed that increasing the concentration of calcium chloride had an inconsistent effect on starch gelatinization. At certain concentrations calcium chloride and copper chloride suppress the gelatinization to below room temperature (Koch and Jane, 2000). Several hypotheses for the mechanisms have been proposed. These include dipole-ion interactions between starch molecules and cations and anions, the viscosity of the salt solution (Jane, 1993), the hydration energy of the salt solution (Gough and Pybus, 1973; Jane, 1993) and the Donnan effect on the granule surface (Oosten, 1982).

Starches from different botanical origins have been shown to interact with salt solutions differently (Medcalf and Gilles, 1966). The actual site on the starch granule where gelatinization begins in the presence of salt is still unknown. However, Jane (1993) reported that potassium thiocyanate and potassium iodide solutions (>2M) gelatinize starch at room temperature from the hilum of the granules to the periphery and was independent of concentration, while in calcium chloride (3M) and lithium chloride (7-10M), starch gelatinization started at the periphery. In a study of the C-polymorph (mixture of A and B-types), Bogracheva et al. (1998), using wide-angle X-ray diffraction technique, showed that the B-polymorph was present in the granule interior while the A-polymorph were located at the periphery. However, the authors reported that during gelatinization of the starch in excess potassium chloride (0.6M) solution, the B-polymorph in the interior of the granule lost crystallinity first accompanied by loss of birefrigence before the A-polymorph in the peripheral region.

2.2.4.1.1.4 Annealing

Thermal treatments can affect the gelatinization characteristics of starch. Annealing and heat-moisture treatments are two common hydrothermal treatments used for modifying physicochemical properties of starch. Both treatments are physical treatments that involve incubation of starch granules at certain moisture content for certain period of time at a temperature above the glass transition temperature but below the gelatinization temperature (Hoseney et al., 1986; Yost and Hoseney, 1986; Jacobs and Delcour, 1998; Tester and Debon, 2000). The main difference between the two treatments is that annealing is carried out in excess water (>60%, w/w) or at intermediate moisture content (40-55%, w/w), while heat-moisture treatment is carried out a low moisture levels (<35%, w/w) [Jacobs and Delcour, 1998; Tester and Debon, 2000]. However, the temperature-moisture conditions for heat-moisture treatments have often been chosen without consideration of the exact gelatinization temperature of the starch at the moisture level being investigated. Therefore, some of the reported results may have been influenced by partial gelatinization (Eerlingen et al., 1996). The change in gelatinization characteristics of starch due to annealing has been interpreted as a partial melting of crystallites with a general rearrangement of the polymer chains in the amorphous region and the remaining crystallites (Marchant and Blanshard, 1978).

The effects of annealing on starch gelatinization was first demonstrated by Gough and Pybus (1971). These researchers annealed wheat starch granules in water at 50 °C for 72 h and using hot-stage polarization microscopy they observed that the treatment caused an increase in gelatinization temperature and a decrease in gelatinization temperature range. Their findings have since been confirmed using DSC experiments (Yost and

Hoseney, 1984; Slade ans Levine, 1987; Tester and Morrison, 1990; Larsson and Eliasson, 1991; Shi and Seib, 1992; Hoover and Vasanthan, 1994b; Eerlingen et al., 1996).

Annealing may increase gelatinization enthalpies (Slade and Levine, 1987; Knutson, 1990; Tester and Morrison, 1990; Hoover and Vasanthan, 1994b; Jacobs et al., 1995; Eerlingen et al., 1996), or remain the same (Yost and Hoseney, 1984; Larsson and Eliasson, 1991; Shi and Seib, 1992; Jacobs et al., 1995; Eerlingen et al., 1996). Russel (1987a, b) found that annealing at 50 °C has no effect on gelatinization enthalpy of waxy maize, but an increase in enthalpy of amylose containing starches. Enthalpy of gelatinization post-annealing cannot be less than for the native starch because annealing leads to elevation of starch gelatinization temperature and sharpening of the gelatinization range (Jacobs and Delcour, 1998; Tester and Debon, 2000, 2000).

Annealing occurs most rapidly and to the largest extent, just below the temperature at which gelatinization starts (Slade and Levine, 1987; Knutson, 1990; Larsson and Eliasson, 1991). It has been reported that multistep annealing allows higher annealing temperature than can be obtained by single step annealing (Knutson, 1990; Eerlingen et al., 1996). Longer incubation time generally increases gelatinization temperatures and enthalpies are more pronounced (Larsson and Eliasson, 1991; Hoover and Vasanthan, 1994b). Krueger et al. (1987) observed slightly larger and significant changes in gelatinization temperature of maize starch with increasing moisture up to 67% (w/w) and no effect thereafter. Hoover and Vasanthan (1994) reported that gelatinization temperatures and enthalpies of wheat, oat, potato and lentil starches increased with increasing moisture content up to 80% (w/w).

Heat-moisture treatment, as for annealing, increases the gelatinization temperature but the gelatinization temperature range remains the same or broadens rather than narrowing as observed for annealing (Hoover and Vasanthan, 1994a; Eerlingen et al., 1996; Hoover and Manuel, 1996). Gelatinization enthalpy has been shown to decrease or remain unchanged on heat-moisture treatment (Hoover and Vasanthan, 1994a; Eerlingen et al., 1996; Hoover and Manuel, 1996).

2.2.4.2 Retrogradation

Retrogradation is the structural transformation that take place when gelatinized starch is cooled and stored. The actual mechanism(s) has not been fully elucidated. However, it is postulated that upon cooling of the gelatinized starch paste, the amylose polymer chains begin to reassociate as double helices. The double helicals can form tightly packed three-dimensional structures that are stabilized by hydrogen bonds between the hydroxyl groups of starch chains (Wu and Sarko, 1978; Eerlingen and Delcour, 1995; Haralampu, 2000; Karim et al., 2000; Hoover, 2001).

Starch retrogradation is a non-equilibrium thermoreversible recrystallization process that is governed by a consecutive three step mechanism of nucleation (formation of double helixes of 40-70 glucose units between the ends of amylose molecules, favouring elongation), propagation (packing of double helical regions of chain folding) and maturation (Slade and Levine, 1987). The kinetics of starch retrogradation exhibit a strong temperature dependence because the nucleation rate increases exponentially with decreasing temperature down to the Tg, while propagation rate increases exponentially with increasing temperature up to the melting temperature (Silverio et al., 2000).

Crystallization can only occur in the temperature range between the Tg and the melting temperature because nucleation and propagation are liquid state events which require orientation mobility of the polymer chains (Silverio et al., 2000). Basically retrogradation involves two steps. The initial step is phase separation, which results in a polymer rich network. While the second phase is the formation of double helices in the polymer rich phase (Gidley and Bulpin, 1989; Clark et al., 1989). For a starch gel containing 50% water, the Tg is approximately -5 °C and the melting temperature is about 60 °C. Slade and Levine (1987) showed that the greatest extent of staling (retrogradation) was attained by nucleation at 0 °C followed by propagation at 40 °C and that the extent of nucleation and overall crystallization in wheat starch-water mixture (1:1) increased with increasing time to nucleation. Heating to temperatures within or above the gelatinization temperature range has been shown to influence the extent of subsequent retrogradation (Fisher and Thompson, 1997). When gelatinized starch is cooled, amylose crystallization occurs quickly to form a gel network contributing to the initial development of gel firmness, while amylopectin recrystallizes much slowly contributing more to the subsequent slow increase in gel firmness (Jane and Robyt, 1984; Miles et al., 1985; Ring et al., 1987; Leloup et al., 1992).

Starch retrogradation has been investigated by a number of procedures including X-ray diffraction, DSC, spectroscopic methods (NMR, Raman, FT/IR) and rheological techniques. Irrespective of the X-ray diffraction pattern in the original starch granule, retrograded starch shows mostly a B-type pattern. Retrograded amylose and amylopectin crystallites have been shown to melt at 120-170 °C (Sievert and Pomeranz, 1989), and 55-70 °C (Eerlingen and Delcour, 1995), respectively. Zeleznak and Hoseney (1986) prepared wheat starch gels containing 15-80% starch, then aged them for seven days at 25 °C before heating in a DSC. The authors reported minimum enthalpies for 15 and 80% gels and maximum enthalpy values in gels of 50-60%. Similar results were reported by Slade and Levine (1987), who postulated that the decrease in enthalpy at high starch concentration could be due to increasing plasticization by water while the decrease at low concentration may be due to a dilution effect.

2.2.4.2.1 Factors influencing starch retrogradation

The rate and extent of retrogradation of starches have been shown to be influenced by starch composition, structure and concentration, storage conditions (temperature and duration of storage), physical and chemical modifications and the presence of other compounds (lipids, sugars and salt). Several researchers have shown that the degree of retrogradation depends mainly on the botanical source of the starch (Silverio et al., 2000). The rate and extent of retrogradation increase with increasing amount of amylose (Russel, 1987a). Generally, cereal starches retrograde to a lesser extent than potato and pea starches. This has been attributed to the shorter average chain lengths in the cereal amylopectin (Silverio et al., 2000). Fredriksson et al. (1998) reported that the weight average degree of polymerization was 26, 33 and 27 for the A-, B- and C-type of starches, respectively. Their results in general showed that potato starch (B-type) exhibited the highest retrogradation enthalphies and cereal starches (A-type) the lowest, while pea starch (C-type) showed an intermediate enthalpy.

Very short chains (6-9 glucose units) retard retrogradation while large amount of medium chains (15 glucose units) and an increasing ratio of A-chains to B-chains promote retrogradation (Würsch and Gumy, 1994; Shi and Seib, 1992; Kalichevsky et al., 1990). It has been postulated by Wurch and Gumy (1994) that the inhibition of retrogradation by short chains may be due to the short external chains hindering the reasssociation of the long external chains. Gidley et al. (1986) reported that a minimum of 8-9 glucose units are required for retrogradation of starch (0.5-3% w/v, starch solution). In addition to starch composition and concentration, retrogradation can be affected by the fine structure of the starch components.

Lipids and surfactants can also influence the rate and extent of retrogradation (Whittam et al., 1986; Eliasson and Gudmundsson, 1996; Fredriksson et al., 1998; Lai et al., 2000). These researchers have shown that in the presence of lipids, emulsifiers and surfactants retrogradation of starch is retarded. For example, Whittam et al. (1986) reported a reduction in amylose gel rigidity when lipids were added in the form of fatty acids (C10-C18) or monoglycerides and the effect was dependent on both lipid concentration and lipid acyl chain length. Eliasson and Ljunger (1988) showed that monoglycerides are more effective than the diglycerides and triglycerides. It has also been shown that the ability of monoglycerides in inhibiting retrogradation increases with a decrease chain length (Huang and White, 1993). The mechanism by which these additives retard retrogradation has not been fully elucidated. However, lipids have been postulated to form helical inclusion complexes between starch molecules (mainly amylose) and lipid hydrocarbon chains, as was shown in Fig 1.5, and could therefore hinder crystallization during gel storage. Surface adhesion of lipids on amylopectin chains or on the starch granule surface may also retard retrogradation (Germani et al. (1983). Hoover (1995) postulated that reduced retrogradation in the presence of lipids could correspond to a decrease in the mobility (due to water-water interaction and/or to an increase in viscosity of starch suspension) of those starch chains involved in double-helix formation and lateral association during gelatinization.

Studies have shown that sugars affect the extent of retrogradation of starch and the melting temperatures of retrograded starch. However, some of the findings are conflicting and the mechanism by which sugars influence retrogradation has not been fully understood. Maxwell and Zobel (1978) observed that starch crystallization in the presence of sugars was in the following order: fructose > glucose > sucrose. But the final rigidity after 5 days of storage was: Control > fructose > glucose > sucrose. However, Germani et al (1983) reported that the rate of retrogradation in the presence of sugars was in the following order: maltose > sucrose > glucose.

Salts (anions and cations) do affect the rate and extent of retrogradation and for most salts increased concentration results in a decrease of starch retrogradation (Hoover, 2000). The exact mechanism by which anions and cations influence retrogradation is unclear. However, using heated starch, Ciacco and Fernandes (1979) showed that retrogradation rates were increased by anions in the order: $I^-<Br^-<Cl^-<F^-$, while the cations increased retrogradation in the order: $K^+<Li^+<Na^+$.

2.2.5 Resistant starch

2.2.5.1 Introduction and definition

Starch present in food has different rates and range of digestion in the human gastrointestinal tract. There can be three categories of starch characterized by differences in the rate of digestion in the small intestine (Englyst et al., 1992). Rapidly digestible starch (RDS) is rapidly and completely digested in the small intestine. This is associated with elevated plasma glucose and insulin and is therefore linked to diabetes, coronary heart disease and the ageing process (Soral-Smietana et al., 2000). Slowly digestible starch (SDS), is slowly digested in the small intestine and has a moderate influence on the plasma glucose and insulin levels. Resistant starch (RS) is not digested in the small intestine.

Resistant starch was originally defined by Englyst et al. (1982) as the starch accessible to amylases only after solubilization with potassium hydroxide or dimethyl sulphoxide. Resistant starch has been defined by EURESTA, as the starch or products of starch degradation that escapes digestion in the small intestine of healthy individuals and may be completely or partially fermented in the colon (Englyst et al., 1992). Previously RS had been categorized into three types according to their degree of resistance to enzyme hydrolysis (Englyst et al 1992). The type-I RS (RS1) is classified as being physically inaccessible to pancreatic α-amylase degradation. They are locked in plant cells, entrapped in a non-digestible (by amylases) matrix (Haralampu, 2000). This fraction can be found in partially milled cereal grains and legume seeds. The type-II (RS2) is the native uncooked (ungelatinized) starch granules of B-type found in foods especially raw potato, unripe banana and high amylose starch. Their high density and semi-crystallinity hinder the entry of amylases into the structure and thus reduce susceptibility to enzyme (Galant et al., 1992). The type-III (RS3) is the fraction that forms after the heat-moisture treatment of starch. These may be present in products containing retrograded starch such as cooled, cooked potatoes and canned peas and beans (Eerlingen and Delcour, 1995).

A fourth type, type-IV (RS4) has been included among resistant starch. Their resistance to amylase digestion has been induced by thermal or chemical modification (Eerlingen and Delcour, 1995). For example, the formation of glycosidic bonds other than α-(1→4) and α-(1→6) by heat treatment, i.e., transglycosidation reduces the availability of starch to amylolytic enzymes (Theander and Westerlund, 1987). Cross-linking or presence of substituents, such as hydroxypropyl, acetyl and succinyl groups, also reduces the digestibility of the starch (Bjork et al., 1989). Since RS3 was the main focus of this study, it will be discussed in detail in the following sections.

2.2.5.2 Resistant starch type III (RS3)

Resistant starch Type III (RS3) has generated a lot of interest in the food industry, mainly due to its nutritional properties and thermal stability. This allows RS3 to be stable in most normal cooking operations and enables its use as an ingredient in a wide variety of conventional foods (Haralampu, 2000). Many investigators have shown that amylose contributes to the formation of thermally stable RS3 complex (Jane and Robyt, 1984: Sievert and Pomeranz, 1989).

During starch gelatinization, amylose is leached out from the granules into solution as a random coil polymer. Upon cooling, amylose chains reassociate as double helical coils and can form tightly packed structures that are stabilized by hydrogen bonds (Eerlingen and Delcour, 1995; Haralampu, 2000). Jane and Robyt (1984) suggested that the amylose aggregates consist of regions of crystalline double helices of about 10 nm interspersed in the amorphous regions. Gidley and Bulpin (1989), Gidley et al. (1995) and Clark et al. (1989) showed that the rate of aggregation depends on the chain length of amylose. Eerlingen et al. (1993b) demonstrated that in an aqueous solution of amylose, the chain length of amylose had no influence on the chain length and the crystalline structure (B-type pattern) of the RS3 obtained. But they did observe that amylose chain length had an impact on the RS3 yield. Due to the similarity in the chain length of the RS3 formed, these researchers (Eerlingen et al., 1993b) proposed two mechanisms (models) for RS3 formation in an aqueous amylose solution: (i) the micelle model, that explains the formation of RS3 by aggregation of different amylose molecules over a particular region of chains and (ii) the Lamellar model, that explains the lamellar structure formation by folding of the polymer chains. The fold zones are amorphous while the center of the lamellar is crystalline. These models are illustrated in Fig. 2.10. RS3 is not a distinct chemical entity but a set of physical states, that is, the formation of RS3 is a physical rather than a chemical phenomenon. This is supported by the observation that RS3 can be digested after solubilization in 2M potassium hydroxide or dimethyl sulfoxide (Berry, 1986).

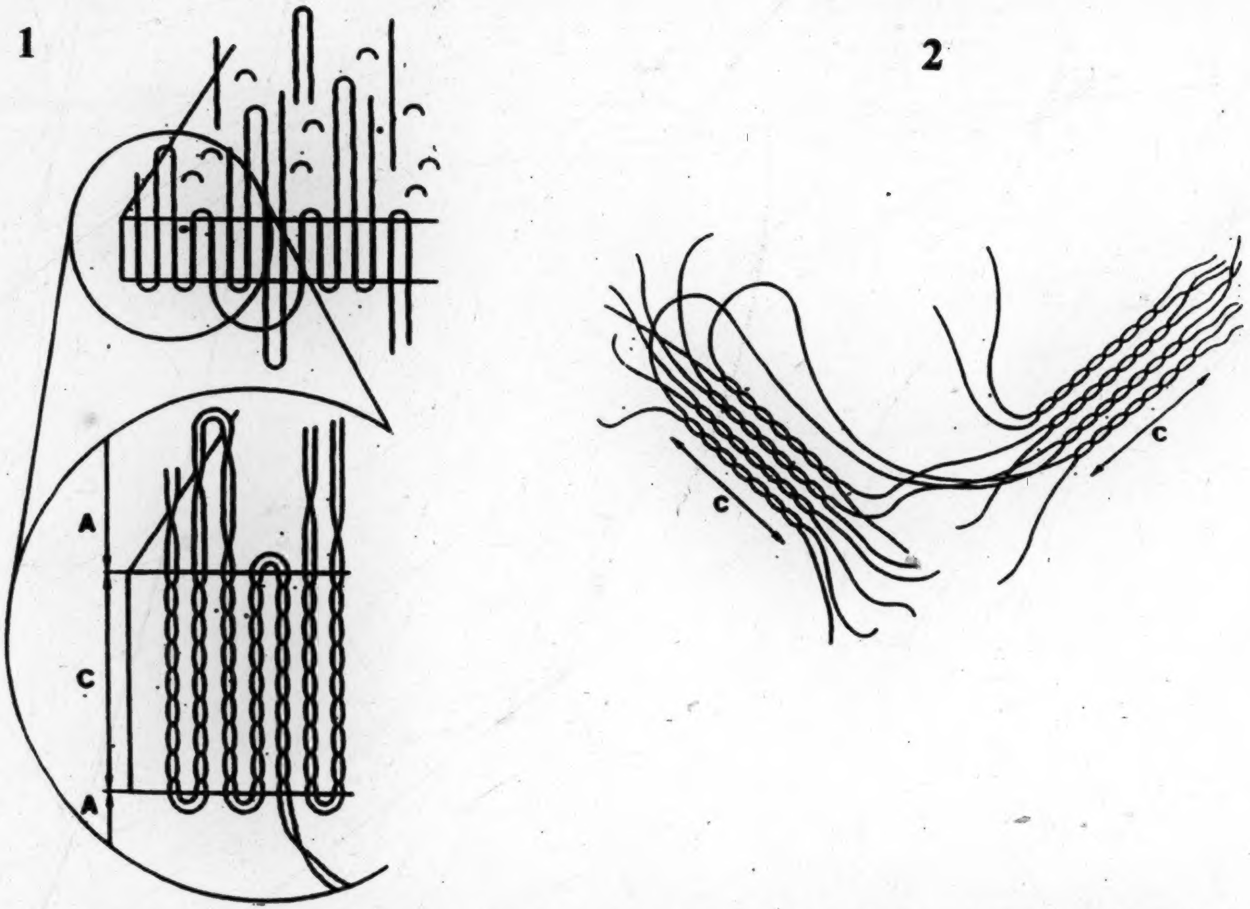


Figure 2.10 Schematic representation of mechanisms involved in RS3 formation in aqueous amylose solutions. (1) Lamella model: Lamellar structures are formed by folding of polymer chains. The fold zones are amorphous (A) while center of lamella is crystalline (C), and (2) Micelle model: double helices are ordered into crystalline structure (C). (adapted with permission from Eerlingen et al., 1993b).

2.2.5.3 Factors affecting RS3 formation

Research has shown that RS3 is formed due to retrogradation of amylose after heating/gelatinization and cooling of the starch. The higher the amount of amylose in the starch the greater is the yield of RS3 expected to form (Berry, 1986; Sievert and Pomeranz, 1990). Amylopectin, because of its branched structure and higher molecular weight, interferes with the retrogradation of amylose (Berry, 1986). The amylose to amylopectin ratio will therefore affect the yield of RS3 formed. The higher the ratio, the greater is the RS3 formation. It has been reported that the amount of retrograded amylose may be increased by removal of amylopectin with a debranching enzymes such as pullulanase or isoamylase (Berry, 1986, Thompson, 2000).

The source of the starch (waxy normal/regular or high amylose) has a profound effect on the yield of RS3 formed. The degree of polymerization (DP) of amylose has an effect on the yield of RS3 formed. Gidley et al. (1989) reported that a minimum DP of 10 is necessary to form the double helix structure in retrograded amylose. The average chain length of RS3 varies between DP 22 and 65 glucose residue (Berry, 1986; Russel et al., 1989). The yield of RS3 formed rises with DP up to 100 and remains unaffected thereafter (Eerlingen et al., 1993b). Water content of the starch affects RS3 formation. A minimum amount of water is necessary for plasticization of the environment, and for nucleation and formation of the crystal structure (Eerlingen and Delcour, 1995). Sievert and Pomeranz (1989) showed that the maximum yield of RS3 was generated when starch:water ratio of 1:3.5 is applied.

Cereal starches contain sufficient monoacyl lipids to form lipid-saturated complexes with about one third of their amylose (Morrison, 1993). Amylose complexed with lysophospholipid (LPL) raises gelatinization temperature while lipid-free amylose has the opposite effect (Morrison et al., 1993b). Presence of complexing lipids reduces RS3 yields. Many investigators have attributed this to the formation of amylose-lipid complex (Morrison, 1988; Czuchajowska et al., 1991). Endogenous or added lipids can form complexes with amylose resulting in less amylose being available to form enzyme resistant double helices.

At high concentration, sugar has an influence on RS3 yield. A decrease in RS3 yield was detected in wheat starch gels containing high levels of sugar while an increase was noted with high amylose corn starch containing added sugar (Eerlingen et al., 1994b). These researchers could not rationalize if the difference in RS3 yield was caused by the added sugar.

Processing conditions have a significant effect on the formation of RS3. It has been shown by many researchers that heating-cooling regimes results in the formation of RS3 (Sievert and Pomeranz, 1989: Vasanthan and Bhatty, 1998). The storage time and temperature also affects RS3 yield and type. Eerlingen et al. (1993b) reported that high temperature (100 °C) and prolonged incubation favoured the formation of resistant A-type crystalline structures whereas the B-type crystals were formed at relatively low temperatures (0 °C and 68 °C). However, most RS3 are of the B-type polymorph.

2.2.5.4 Health benefits of RS3

Studies involving both rats (Ranhotra et al., 1991; Muir et al., 1994; de Deckere et al., 1995) and human beings (Muir et al., 1995) have shown that RS3 escapes digestion in the small intestines and is slowly fermented in the large intestine. In the small intestine

RS3 has been associated with increased mal-absorption of starch, resulting in decreased postprandial glucose and insulin response (Raben et al., 1994). Modulation of glucose release and uptake in humans can be an important consideration in the use of RS3 in food products. This has significant implications for RS3 in food formulation for certain target groups such as diabetics and athletes. Digestion of RS3 may occur over 5-7 h period (Raben et al., 1994). In the colon RS3 has been shown to increase faecal bulk and lowers the colonic pH (Haralampu, 2000). It has been proposed as a potential prebiotic and therefore has the potential of improving health (Conway, 2001). The portion of RS3 fermented in the colon produces a wide range of short chain fatty acids (SCFAs) including acetate, propionate and butyrate (Brown et al., 2000). The SCFAs has positive impact on the bowel health including increased absorption of magnesium and calcium, epithelial cell proliferation, balance of bacterial species and bacterial metabolism of bile salts (Lopez et al., 2001). Resistant starch may also function as a culture protagonist because it provides enhanced bacterial survival when combined with probiotics (Conway, 2001). Therefore RS3 physiologically behaves similarly to soluble fermentable fiber such as β-glucan, guar gum, etc. but assay as insoluble fiber. Some findings have not been very conclusive. For example, it has been reported that RS3 has cholesterol lowering effects in rats (Ranhotra et al., 1996a, b), but Heijnen et al. (1996) did not find this effect in human studies with normolipidemic subjects.

In humans, digestibility of RS3 is reduced by factors that can be broadly categorized into two groups, intrinsic and extrinsic factors. The intrinsic factors are all the factors that make the different types of RS3 indigestible in the small intestine, that is, physical inaccessibility by the enzymes, compactness of the granules, retrograded amylose or modified starch. Extrinsic factors include food particle size, transit time through the colon and the viscosity of the meal that affect the diffusion of the enzymes (Eerlingen and Delcour, 1995). Most studies indicates that 30-70% of the RS3 is metabolized, while the balance is excreted in the faeces. (Ranhotra et al., 1996c; Behall and Howe, 1995, 1996; Haralampu, 2000).

Resistant starch intake can be increased by consumption of foods rich in RS3, enrichment of foods by isolated RS3, inclusion of new ingredients such as high amylose starch in the food formulations, and simply by increasing the intake of starchy foods (Wisker, 2000). Traditional fibers (i.e bran, cellulose, β-glucan, pectin, etc.) tend to increase the firmness of baked goods. It also limits the expansion of extruded products and negatively influences its texture. But it has been clearly demonstrated that RS3, which also assays as total dietary fiber (TDF), can be added at high levels while minimally interfering with the food structure (Haralampu, 2000). A need exists, therefore, to develop foods that are high in RS3 content.

2.2.5.5 *In vitro* quantification of resistant starch

Several *in vitro* procedures for the quantification of RS3 have been used or proposed. Most of these procedures can be placed into two major groups. One group determines total starch without the use of dimethyl sulfoxide (solubilizes the enzyme undigestible starch and render it digestible) and the residue left is hydrolyzed with dimethly sulfoxide and the starch in this fraction is referred to as resistant starch. The other group runs two parallel determinations one with and the other without dimethyl sulfoxide. The difference in starch content between the two parallel determinations is considered to be the resistant starch. The enzymes used and the time-temperature conditions of enzyme reaction vary widely leading to discrepancies in the amount obtained using different procedures

There is no regulatory definition of RS in North America and RS is, therefore, typically quantified as part of the total dietary fiber (TDF). The most commonly used procedure is the Prosky method which is identified as AOAC 991.43 (AOAC, 1997) or AACC 32-07 (AACC, 2000). In this procedure, starch digestion is carried out with thermostable α- amylase at 95-100 °C. The Megazyme procedure (amyloglucosidase/α-amylase method) for total starch determination also utilizes high temperatures during the initial stage (Megazyme International Ireland Ltd., 1997). The high temperature may melt some of the RS components (i.e RS1 and RS2) leading to underestimation of resistant starch.

In the Englyst et al. (1982) procedure the samples are first heated in a sodium ethanoate buffer (pH 5.2) for 60 min at 100 °C to gelatinize the starch. The samples are then incubated overnight at 42 °C with amylolytic enzymes, hog pancreatic α-amylase and pullulanase (a debranching enzyme). Samples are then treated with amyloglucosidase and the amount of glucose determined by glucose peroxidase assay. Berry (1986) modified the method of Englyst et al. (1982) by omitting the initial heating step at 100 °C and included a potassium hydroxide solubilization step and used an enzyme assay kit. Bjorck et al. (1986) proposed a different procedure. In their method the samples are incubated with a thermostable α-amylase in boiling water bath for 15 min. The samples were then digested with amyloglucosidase (pH 4.75 for 30 min at 60 °C). Pepsin was used to digest protein but no difference was observed with or without this enzyme in bread products. However, pepsin increased the starch hydrolysis in raw wheat flour.

A collaborative inter-laboratory study (Champ, 1992) comparing the RS determination methods of Berry (1986) and Bjorck et al (1986) demonstrated that the procedure involving incubation of the samples with pancreatin at 37 °C for 16 h (Berry, 1986) resulted in higher RS yields for almost all the samples than the procedure where the heat-stable α-amylase was heated to 100 °C (Bjorck et al., 1986). When applying temperatures of up to 100 °C starch gelatinizes and therefore no proper quantification of true RS (RS1, RS2 and RS3) is made. It has been shown that the melting temperature of retrograded amylopectin is 55–70 °C while that of amylose-lipid complex is 90–110 °C (Eerlingen and Delcour, 1995).

Different enzymes have been used in the *in vitro* determination of RS3. Alpha-amylase is commonly used singly or in combination with amyloglucosidase or pullulanase. Bacterial α-amylase has an optimum pH of 6.0 and temperature of 90 °C while those of human pancrease are pH 6.9 and 50 °C, respectively (Colonna et al., 1992). The source of the enzyme will therefore have an effect on the degree of hydrolysis of starch. Some researchers have also used proteolytic enzymes to liberate physically inaccessible starch and to remove amylolytic enzymes (Siervert and Pomeranz, 1989; Siljestrom et al., 1989).

An analytical procedure has been proposed by Englyst et al. (1992) for determining rapidly digestible starch, slowly digestible starch and resistant starch. The method further fractionates the RS1, RS2 and RS3. Due to the tediousness and time consuming nature of this procedure, it has not been widely adopted. But they did demonstrate that the rate of starch hydrolysis is mainly determined by amylase activity and is not significantly influenced by the presence of amyloglucosidase that degrades α-limit dextrins left by amylase to glucose (Englyst et al., 1992). Amyloglucosidase (EC 3.2.1.3) is able to hydrolyze α-(1→4), α-(1→6) and α-(1→3) linkages to produce glucose from dextrin. Invertase is incorporated into the procedure to convert all sucrose present into its constituent monomers. None of the enzymes used was able to hydrolyze inulin, a fructooligosacharide.

In vitro RS quantification involves determination of RS outside a living organism while *in vivo* methodology involves using ileostomy subjects or the intubation procedure. Ideally *in vitro* RS quantification values should be as close as possible to the *in vivo* data which measure the amount of starch that is physiologically significant as a dietary fiber. McCleary (2001) proposed that incubation temperatures for α-amylase be in the range of gelatinization temperature for most normal starches in order to realize RS values more in line with the *in vivo* data. Recently, McCleary and Monaghan (2002) developed an *in vitro* procedure for RS determination that gives values close to the *in vivo* data. Quantification data that give *in vitro* values that are as close as possible +to *in vivo* data are very useful in physiological studies on the effect of RS. However, for the development of RS3 ingredients to be incorporated into novel food products containing high TDF, a procedure to quantify RS3 that survive the normal cooking methods needs to be developed. RS3, as discussed above, is the most heat-stable of the RS types and is likely to maintain its structural features during normal cooking temperatures.

2.2.6 *In vitro* amylolysis of starch

2.2.6.1 Introduction

In vitro enzyme hydrolysis of starches have been used in simulation studies to investigate the effects of different food components on digestibility of starch *in vivo*. Alpha-amylase (α-(1→4)-D-glucan glycanohydrolase, EC 3.2.1.1) is most often an endo-splitting enzyme that hydrolyses the α-(1→4)-glycosidic bonds in amylose, amylopectin and related oligosaccharides in a more or less random fashion (Whitaker, 1984). The splitting is theoretically entirely random, however, the action patterns of a number of α-amylases, particularly from microorganisms, show consistent and reproducible product pattern characteristics of an α-amylase from a particular source (Robyt and French, 1963). The molecular weight of the α-amylases are in the range of 50,000 and all contain one gram atom of Ca (II) per molecule. The activities of the mammalian and perhaps some of the bacterial α-amylases are stimulated by halogen ions. The role of Ca (II) in α-amylase is to maintain the secondary and tertiary stability of the molecule. There is no evidence so far to indicate that Ca (II) plays a direct role in binding or transforming the substrate (Whitaker, 1984). By iodine binding studies, Colonna et al. (1988) showed that there is no preferential hydrolysis of either amylose or amylopectin by the α-amylases.

2.2.6.2 Enzyme kinetics of α-amylase

Hydrolysis of starch by α-amylase involves an enzyme in solution acting on a solid substrate. The efficiency of absorption of the enzyme and the surface area accessible by enzymes are critical kinetic parameters (Bertoff and Manelius, 1992).

Alpha-amylase hydrolysis of various starches proceeds rapidly initially followed by a progressively slow or constant hydrolysis. The two-stage hydrolysis is more pronounced at high enzyme concentrations (Bertoff and Manelius, 1992; Planchot et al., 1995; Kimura and Robyt, 1995). The initial hydrolysis rates are similar in waxy and normal maize, large granules of barley and wheat, but their initial hydrolytic products differ (Bertoff et al., 2000). Waxy starches are usually hydrolysed faster than normal starches (Leach and Schoch, 1961; MacGregor and Balance, 1980; Bertoff and Manelius, 1992). In general, most cereals and cassava (A-polymorph) are more readily hydrolysed than amylomaize and potato (B-polymorph) starches (Gallant et al., 1992; Planchot et al., 1995; Oates, 1997). It has been shown that there is a higher resistance towards α-amylase and glucoamylase for high amylose starches compared to normal and waxy starches in maize and barley (Kimura and Robyt, 1995; Planchot et al., 1995)

The compositional and structural features of the starch determine the rate and extent of action of a given enzyme. These features vary among and within species and includes amylose-amylose and amylose-amylopectin interchain associations (Leach and Schoch, 1961; Vasanthan and Bhatty, 1996), amylose-lipid complex (Anger et al., 1994, Morrison, 1995; Appelqvist and Debet, 1997; Lauro et al., 1999), and type and degree of crystallinity (Gerard et al., 2001). It can also be influenced by the physical (e.g., gelatinization and retrogradation) and chemical modification the starch may have undergone prior to enzymatic hydrolysis (Tharanathan and Ramadas Bhat, 1988; Lauro et al., 1993; Kurakake et al., 1996; Wolf et al., 1999; Manelius et al., 2000). It has been shown that lipid-complexed amylose is less susceptible to α-amylolysis than free amylose and amylopectin (Lauro et al., 1999).

Waxy starch from barley is more susceptible than normal barley starch to degradation by α-amylase (Goering and Eslick, 1976) and glucoamylase (Fuwa, 1982). Conflicting reports have been published on the susceptibility of high amylose barley starch to enzymatic degradation. Earlier reports claimed that high amylose barley starches were hydrolysed more quickly than the corresponding normal starches by α-amylases from several sources (Merritt, 1969). More recent reports claim that high amylose barley starch is more resistant (Ellis, 1976) or equally resistant (Bjorck et al., 1990) to hydrolysis by amylases than normal barley starch. Recently, Li et al. (2004) showed that normal barley starch was more susceptible to both *Bacillus subtilis* α-amylase (BAA) and *Aspergillus niger* amyloglucosidase (AAG). However, with porcine pancreatic α-amylase (PAA), the susceptibility of high amylose starch was initially slightly higher, but after 20h the normal barley starch hydrolysis started to exceed that of high amylose starch. The rate and extent of hydrolysis of a given starch will depend on the enzyme source and hydrolysis conditions. The hydrolytic conditions may include the enzyme concentration, pH, temperature and ion concentration (Blakeney and Stone, 1985; Franco and Ciacco, 1987; Manelius and Bertoff, 1996; Textor et al., 1998). The degree of hydrolysis of PAA after 72 h reached 91-97% in normal, waxy and high amylose barley starches (Li et al., 2004). However, BAA and AAG showed significantly lower degress of hydrolysis in normal (<30%) and high amylose (<37%) starches than in waxy HB starch.

2.2.6.3 Alpha-amylase hydrolytic products

The initial major products of α-amylase hydrolysis are the dextrins and the final major products are maltose and maltotriose (Whitaker, 1984). However, a number of studies have shown that α-amylase from different sources results in different hydrolytic products. Porcine pancreatic α-amylase (PAA) hydrolytic products are mainly maltose, maltotriose and maltotetraose (Robyt, 1984), while α-amylase from *Bacillus amyloliquefaciens* (BAA) produces mainly maltotriose, maltohexaose and maltoheptaose (Yook and Robyt, 2002). An α-amylase from *Aspergillus fumigatus* produced glucose as the only end product (Planchot et al., 1994). Despite the differences in end products of α-amylases from different sources, all hydrolytic products from α-amylase hydrolysis of starch have been shown to possess initially α-anomeric configuration of the substrate (Kuriki and Imanaka, 1999).

Maltose is not hydrolysed by α-amylase but does act as a competitive inhibitor. Maltose can bind to the active sites of α-amylase but the binding does not position the α-(1→4)-glycosidic bond properly with respect to the catalytic groups so that it can be hydrolysed (Whitaker, 1984). The major end products (glucose, maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose) from the hydrolysis of starch by α-amylases from different sources may be due to the structural barrier of α-amylase at the active site preventing attack on the α-(1→4)-bonds next to the branch points (Reilly, 1985; Nigam and Singh, 1995). It has also been reported that the oligosaccharides formed may cause gradual reduction in the rate of amylolysis by inhibiting α-amylase activities (Leloup et al., 1991; Colonna et al., 1992). These researchers postulated that the increase in maltose and maltotriose concentration may change the molar ratio of the oligosaccharide to enzyme and therefore influence the absorption equilibrium by forming oligosaccharide-enzyme complex.

Elimination of soluble hydrolytic products by dialysis increased the degree of hydrolysis (Franco and Ciacco, 1987). When amyloglucosidase is used in combination with α-amylase, the amyloglucosidase hydrolyses maltose and maltotriose into glucose and therefore, increased the rate and extent of α-amylolysis (Colonna et al., 1992; Liakopoulon-Kyriakides et al., 2001).

2.2.6.4 Mechanism of action of α-amylase

The mechanism of action of α-amylases has not been clearly or fully elucidated. However, Robyt and French (1970) showed that α-amylase hydrolyses starch by a multiple attack mechanism. The direction of the multiple-attack is from the reducing end towards the non-reducing end. Once the enzyme forms a complex with the substrate and produces the first cleavage, the fragment with the non-reducing end dissociates from the active site while the fragment with the newly formed hemi-acetal reducing-end remains attached to the active site and repositions itself to cause another cleavage and the formation of maltose and maltotriose (Robyt, 1984).

X-ray diffraction studies have shown that PAA and human α-amylase contain 5-11 subsites for sugar residues to ensure maximum binding (Robyt and French, 1963; French and Robyt, 1970; Robyt, 1984). The active site of PAA has been shown to contain five subsites with catalytic groups between the second and third subsites from the reducing-end subsite, while BAA contains nine subsites in which the catalytic groups are positioned between the third and fourth subsites from the reducing-end subsites (Robyt and French, 1970, Robyt, 1984). Alpha-amylase also possesses a starch binding domain remote from the active site which is probably important for holding the α-amylase onto its insoluble substrate (Slaughter et al., 2001). Scanning electron microscopy and transmission electron microscopy have been used to follow the action patterns of various starch types and different enzyme sources. The action patterns of α-amylases are similar between cereal starches but differ in some tuber starches (Gallant et al., 1972, 1992; Valetudie et al., 1993; Planchot et al., 1995). However, enzymatic action patterns were shown to be different between bacterial and PAA in yam starch (Valetudie et al., 1993).

2.3 BETA-GLUCAN

2.3.1 Introduction

The β-glucan contents of barley ranges from 4-6% in normal barley and 6-11% in waxy hull-less barley (Fastnaught et al., 1996; Bhatty, 1999). However, some waxy hull-less barley cultivars (e.g Arizona variety) and waxy hulled-barley (e.g., Prowashonupana) contain β-glucan up to 11% and 17.4%, respectively (Miller and Fulcher, 1994; Bhatty, 1999). Waxy hull-less cultivars generally have higher levels of β-glucan contents than normal cultivars (Ulltrich et al., 1986; Fastnaught et al., 1996). In mature barley grain, β-glucans are found mainly in the endosperm cell wall, where it accounts for about 75% by weight while arabinoxylans and proteins account for the remaining 20% and 5%, respectively (Fincher and Stone, 1986). In addition to genetic factors, the level of β-glucan content in barley is affected by environmental factors. It has been shown that the major environmental factor that influences β-glucan levels appear to be the availability of water during grain maturation. Dry conditions before harvest result in high β-glucan levels while moist conditions causes a decrease in β-glucan (MacGregor and Fincher, 1993).

2.3.2 Composition and Structure

Barley β-glucan consists of linear homo-polysaccharide chains of β-glucosyl residues polymerized through β-(1→3) and β-(1→4) linkages. It has a degree of polymerization of up to 1400 glucosyl residues containing approximately 70% (1→4)-linked β-glucosyl residues and 30% (1→3) linked residues (Woodward et al., 1983).

Molecular weight values for water-soluble barley β-glucans range from 2×10^4 to 4×10^7 Da, depending on the method used for its determination (Fincher and Stone, 1986). The apparent discrepancies in the molecular weight estimates of cereal β-glucans may be attributed to the variation in cell wall structures (thicker cell walls show greater resistance to extraction of high molecular weight polymer), different extraction and isolation protocols used (solvent and temperature affect solubilization), aggregation phenomena (dependent on the structural features and solvent quality) and depolymerization events (endogenous or microbial β-glucanases from contaminating microorganisms) taking place during the extraction step (Izydorczyk and Biliaderis, 2000).

The proposed detailed structure of β-glucan is shown in Fig.2.11. It comprises mainly cellotriosyl and cellotetraosyl units. The β-glucan structure is somewhat similar to cellulose, however, cellulose contains only β-(1→4) linkages that tend to form compact crystalline regions making it water insoluble. In contrast, β-glucan has short β-(1→4) linked chains made up of mostly 2-3 glucose units connected by β-(1→3) glycosidic linkages. Longer chains of (1→4)-β-glucan tends to behave like cellulose and may precipitate out of solution if these chains are longer than 6-7 units (Woodward et al., 1983). β-Glucan chains of up to 14 units of (1→4) linked glucose units have been reported (Cui and Wood, 2000; Vaikousi et al., 2004). Random distribution of the cellotriosyl and cellotetraosyl residues in the polymer chain, together with presence of about 10% by weight of longer chain blocks results in the irregular spacing of the (1→3) linkages ('kinks') in the molecule making them fairly soluble in water (Woodward et al., 1983).

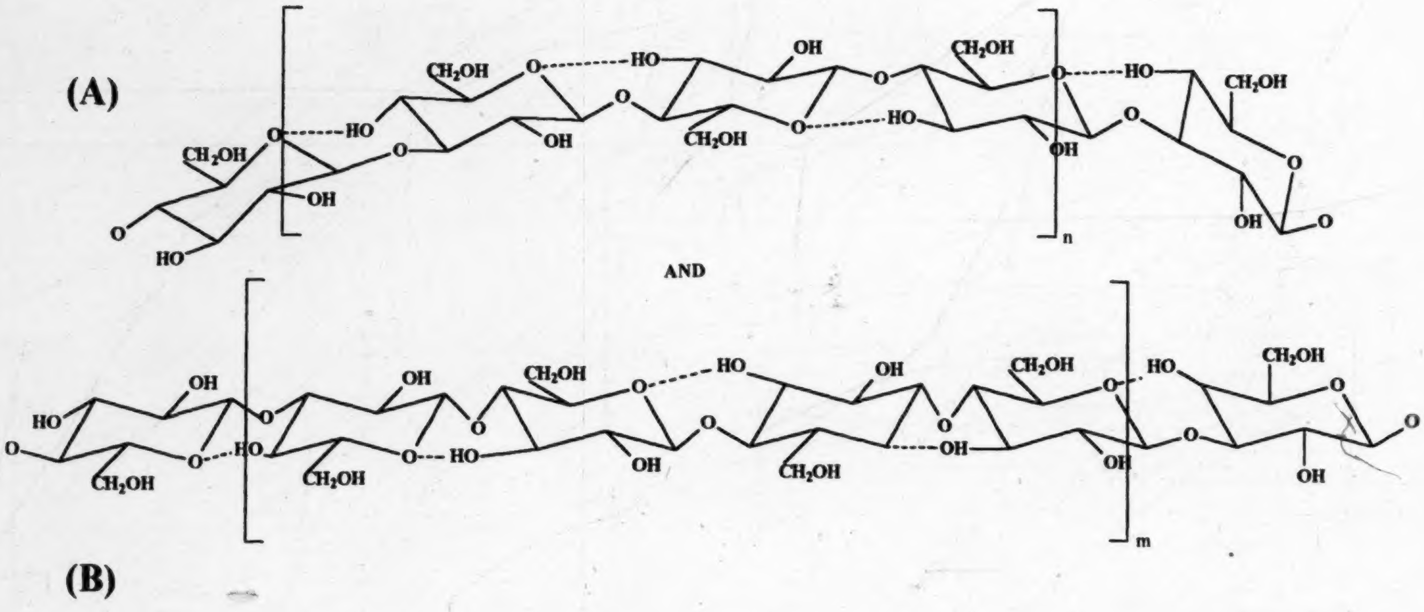

(A)
AND
(B)
CH₂OH
OH
HO
O
n
m

Figure 2.11. The structure of beta-glucan (A) cellotriosyl and (B) cellotetraosyl units.

2.3.3 Rheological properties

2.3.3.1 Viscosity

Beta-glucans are not highly hydrated molecules and, therefore the high intrinsic viscosities of β-glucans in solution may be attributed mainly to their molecular asymmetry, in combination with their molecular weight (Woodward et al., 1983). Beta-glucans in solution have been shown to exist in two states: molecularly dispersed with normal viscoelastic flow behavior and as an infinite network structure (Bohm and Kulicke, 1999a). Solutions of β-glucans have been found to display shear-thinning behavior over shear rates of 1-1500 s^{-1} (Carrier and Inglett, 1999) and the viscosity decreases with increasing shear rates.

The shear thinning behavior is dependent on the molecular weight and concentration of the β-glucan preparations (Burkus and Temelli, 1999; Skendi et al., 2003). Several researchers (Wood, 1984; Autio et al., 1987; Wilkstrom et al., 1994; Dawkins and Nnanna, 1995; Doublier and Wood, 1995; Zhang et al., 1998; Skendi et al., 2003; Lazaridou et al., 2003) have also reported that β-glucans display this shear thinning, pseudoplastic behavior. This behavior can be described by the Power law model

$$S=cR^n ,$$

where, S is the shear stress (NM^{-2}), R is the shear rate (S^{-1}) and c and n are constants for the consistency coefficient and flow behavior index (a measure of departure from Newtonian behaviour), respectively. However, the Power law relationship has a limited shear rate range of validity and thus the measurement should be limited, at most to two decades of shear rate (Doublier and Wood, 1995). The smaller the value for flow behavior index the greater the shear sensitivity. Autio et al. (1987) showed that an increase in β-glucan concentration causes the consistency coefficient to increase and the flow behavior index to decrease, indicating that the solution becomes more pseudoplastic. Pseudoplasticity appears to be important in contributing to good sensory qualities (e.g., mouthfeel and flavor release to foods) and is also important in the pourability of suspensions and emulsions (Dawkins and Nnanna, 1995). Gums with lower flow behavior index (n) have a cleaner mouthfeel, while gums with high n values tend to feel slimy in the mouth (Szczaniak and Farkas, 1962).

2.3.3.2 Gelation

Beta-glucan can form a gel under different conditions and the gelation behavior of β-glucan is controlled by a number of parameters. The two main parameters are molecular weight and concentration. Low-molecular weight gums are more prone to gelation and tend to gel rapidly (Doublier and Wood, 1995; Burkus and Temelli, 1999; Bohm and Kulicke 1999b; Skendi et al., 2003; Lazaridou et al., 2003, Vaikousi et al., 2004). This has been attributed to the smaller chains (low molar mass) being more mobile enhancing diffusion and lateral association (Bohm and Kulicke, 1999b). The concentration determines the segment density in solution and therefore the probability of contact between the coils, which is a basic requirement for three-dimensional network formation. Gel-forming polysaccharides must have a moderately irregular structure that allows partial but not overall association, which may cause precipitation or insolubility. Burkus and Temelli (1999), Doublier and Wood (1995) and Morgan and Offman (1998) reported that high viscosity/high molecular weight β-glucan does not gel and is not influenced by gelation time or concentration. Whereas, Skendi et al. (2003) and Vaikousi et al (2004) have reported that, both low- and high-molecular weight β-glucan fractions ($70\text{-}250\times10^3$ Da) can form gels. However, this discrepancy among researchers could be attributed to the differences in the molecular weight assigned to the high molecular weight β-glucan fractions and experimental conditions.

The molecular structure of β-glucan has been described in detail by a number of researchers. However, the configuration of the molecules in a three-dimensional space necessary for the formation of micelles or gels has not been fully elucidated and therefore the actual mechanisms of how β-glucan chains aggregate and gel are not fully understood. There are a number of theories and postulations that have been considered. Previously a model developed by Fincher and Stone (1986) was the most popular. According to this model the cellulose-like segments of more than three contiguous (1→4)-linked β-glucosyl units are likely to serve as centers for aggregation of the polymeric β-glucan chain. The present consensus is that the straight chain (1→4) cellulosic regions may align and form microcrystalline regions with strong hydrogen bonds. It is due to the length of these cellulosic regions (junction zones) that β-glucans are able to associate with each other to form gels or create micelles. More recently, Bohm and Kulicke (1999a) have proposed a different model. These authors suggested that association of consecutive cellotriosyl units linked by β-(1-3) bonds may be responsible for the formation of extended junction zones leading progressively to a helical structure (Fig. 2.12). They suggested that it is the sections of consecutive cellotriosyl units that constitute the cross-links (junction zones) and gelation of these polysaccharides.

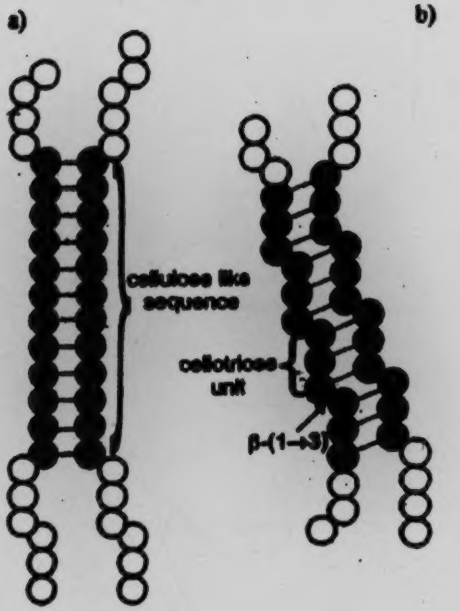

a)
b)
cellulose like sequence
cellotriose unit
β-(1→3)

Figure 2.12. Chain interactions in (1→3)(1→4)-β-glucan leading to gels (a) The common model; sequences of consecutive (1→4)-linkages stick together and (b) model proposed by Böhm and Kulicke (1999a); association of consecutive cellotriose units linked by β-(1→4) bonds which probably forms a helical structure (adapted with permission from Böhm and Kulicke, 1999a).

A similar observation was made by Zhang et al. (1997). The existence of these consecutive sequences of β-(1→3) linked cellotriosyl is yet to be fully elucidated. However, the results of Vaikousi et al. (2004) agrees with this model, when they reported that the samples with the highest cellotriosyl:cellotetraosyl ratios and with lowest molecular weight ($70x10^3$) tended to gel rapidly, though it exhibited the lowest contents of DP5-DP14 which suggested that gelling capacity does not arise from the cellulose-like sequences in the β-glucan chain. The gelling ability of cereal β-glucans have been shown to follow the order wheat > barley > oat, a trend corresponding to the ratio of their tri- and tetra-saccharides (4, 3 and 2, respectively) [Cui and Wood, 2000, Wood, 2002].

2.3.4 Functional properties of β-glucans

Beta-glucans, due to their gelling capacity and ability to increase the viscosity of aqueous solutions, may find uses in the food industry as a natural hydrocolloid and a food thickener. However, the commercial use of β-glucan at a large scale is not common place. As noted by Burkus (2001), the cost of β-glucan may be limiting its large-scale utilization at the moment.

2.3.4.1 Fat substitute

Barley β-glucan gum has been incorporated into breakfast sausages as a fat replacer at a pilot plant level (Morin, 2001). Oatrim®, an extract from α-amylase hydrolysed oat flour containing 1-10% β-glucan has been commercially incorporated into numerous food products. It is used as a fat replacer/mimetics in the development of reduced calorie foods (Inglett, 1990b) and in certain types of low fat milk products

(Pszczola, 1996). Oatrim® can also be used in baked goods, dairy products such as skim milk, meats, sauces, dressings, and soups (Colby, 2004).

2.3.4.2 Foam and emulsion stabilizer

Hydrocolloids are added to foams and emulsions in order to increase the viscosity of the continuous phase, thus decreasing bubble and droplet movement, slowing down drainage and creaming, and resulting in more stable foams and emulsions (Burkus, 2001). Cereal β-glucans can, therefore, be used as a foam and emulsion stabilizer. Data reported by Burkus and Temelli (2000) showed that medium viscosity (20.5 mPa s) pilot plant gum and high viscosity (683.0 mPa s) laboratory gum alone at 1 or 2% (w/w) did not result in foams, and did not stabilize emulsion when applied alone at 1% (w/w). However, when whey protein concentrate was used as an emulsifier and foaming agent, the addition of these β-glucan gums increased ($P \leq 0.05$) the stability of foams and emulsions.

2.3.4.3 Thickening agent

Many researchers have proposed that due to its high viscosity in aqueous solution, β-glucan can be used commercially as a thickening agent (Wood, 1984; Autio et al., 1987, 1992; Bhatty, 1993). However, very little information is available in the literature, which show or demonstrate the use of β-glucan as a thickening agent. This may be because most of the formulations are done by the food industry where the results are most often kept confidential and are rarely reported in scientific journals. Nevertheless, the studies done by Burkus and Temelli (1999), showed that long-term stability of β-glucan solution on its own is very limited. Further research on the thickening properties of β-glucan in traditional and novel foods is necessary in order to compare its performance with other polysaccharides (e.g., galactomannan) that have demonstrated thickening properties in commercial food products.

2.3.5 Effect of food components on the rheological properties of β-glucan

Most food preparations are complex mixtures of various components contributing different functional and nutritional properties. The compatibility of β-glucan with other macromolecules in a food system is important for successful marketing and sustainability of the particular food. There is a dearth of information on the mechanism of interaction of β-glucan with food components such as starch, protein, and gums. The present knowledge in the area is reviewed below;

2.3.5.1 Starch

The effect of starch on the viscosity of barley β-glucan gum was investigated by Burkus and Temelli (1998). The authors reported that the viscosity of β-glucan (0.5%, w/w) in the presence of starch (0.5%, w/w) increased by about 2.5-fold. However, the gum mixture separated into two layers following storage at 4 °C. Prehydration of the starch solution slowed down the separation of the phases. They postulated that the hydrophobicity of the interior of the hydrogen-bonded regions of amylose and β-glucan may have been responsible for such destabilization.

2.3.5.2 Proteins

When barley β-glucan was mixed with milk protein components (whey protein concentrate) a synergistic effect was observed, resulting in higher viscosity (Burkus and

Temelli, 1998; Bansema, 2000). However, the mixture was unstable as the milk proteins were precipitated out. The former authors showed that precipitation could be prevented by the addition of calcium chloride or by increasing the concentration of the gum.

2.3.5.3 Gums

The interaction of oat β-glucan with xanthan, guar and locust bean gum was studied by Nnanna and Dawkins (1996). They reported that oat gum had a synergistic effect with locust bean gum and to a lesser extent with xanthan gum at a total concentration of 0.5% (w/w).

2.3.6 Effect of cooking on β-glucan

Few studies have been conducted to investigate how molecular weight and viscosity of β-glucans are influenced by the various cooking methods areas (Beer et al., 1997; Van der Sluijs et al., 1999). The effects of cooking and storage on the amount and MW of β-glucan in oat bran muffins and oat porridge were investigated by Beer et al. (1997). They reported that β-glucan extracted from muffins had a lower peak molecular weight (MW) when compared to β-glucan of the original brans, but the amount of β-glucan extracted increased. Baking led to a lower MW resulting in an increase in the solubility of β-glucan. The amount of extractable β-glucan in the muffins declined during storage at –20°C. However, there was no change in peak MW of the extracted β-glucan detected during storage. Cooking the oat bran as porridge, did not change the amount of extractable β-glucan compared to the uncooked bran, and the MW remained unchanged.

However, rolled oats cooked as porridge showed a slightly increased extractability relative to the bran.

The effect of different cooking methods (boiling and baking) on pudding containing Oatrim® as assessed by glucose, insulin and glucagons responses was investigated by Van der Sluijs et al (1999). All Oatrim® treatments gave a significantly lower response from that of standard glucose solution. The mean glucose and insulin responses were not significantly different among the different cooking methods but there was a significant difference for glucagons. For glucagon response, the uncooked pudding-containing Oatrim® (control) was significantly higher than boiled and baked puddings while baked and boiled were not significantly different from each other.

2.3.7 Health benefits of β-glucan

The health promoting effects of cereal β-glucans, particularly from oats, have been extensively studied and have been reviewed by Newman et al. (1989), Newman and Newman (1991), Bhatty, (1999), and Malkki and Virtanen (2001). A number of *in vitro*, *in vivo* and clinical studies have confirmed some of these health benefits, specifically plasma cholesterol lowering and attenuation of plasma glucose and insulin. This led to the approval by the FDA (1997) of a health claim for food products containing β-glucan from an oat source (bran, groats, rolled oats), which guarantees ≥0.75 g of β-glucan per serving. These benefits are briefly summarized below.

High serum cholesterol is recognized as a significant risk factor for coronary heart disease. Oat and barley fiber or their fractions have lowered elevated blood cholesterol in both animal trials (Klopfenstein and Hoseney, 1987; Fadel et al., 1987; Newman et al., 1992; Kahlon et al., 1993; Yokoyama et al., 1995; Hecker et al., 1998) and human clinical studies (Anderson et al., 1984, Newman et al., 1989; Braaten et al., 1994; Lupton et al., 1994). These hypocholestrolemic effects of oats and barley have been attributed to their β-glucan contents. However, the actual mechanism on how this is achieved has not been fully understood, but it appears that the viscous consistency of diets containing β-glucan reduces absorption and reabsorption of lipids, bile acids and their metabolites, influencing the plasma cholesterol levels (Kahlon et al., 1993).

The inability of the body to maintain normal glucose levels or to require excessive levels of insulin to do so has been called glucose intolerance, impaired glucose tolerance and insulin resistance. These conditions are associated with obesity and may be preliminary steps in the progression of type 2 diabetes mellitus (Hallfrisch and Behall, 2000). Diets containing oats or barley β-glucans have been shown to lower postprandial glucose and insulin levels (Wood et al., 1994; Wood, 1995; Yokoyama et al., 1997; Bourdon et al., 1999; Hallfrisch and Behall, 2000; Keagy et al., 2001). The reduction of glucose and insulin levels are dependent on both the molecular weight of the β-glucan and the dose ingested (Wood et al., 1994). The actual mechanism is not fully understood but may be related to reduced gastric emptying or due to slower absorption because of the viscosity-related effects of the β-glucan on the intestinal surface area (Yokoyama et al., 1997).

Other health benefits that have been attributed to β-glucans are immunostimulating effects and anticarcinogenic effects. Estrada et al. (1997) reported that high viscosity and high molecular weight oat β-glucan had an immunostimulating effect *in vitro* and *in vivo* in mice subjected to intraperitoneal administration that increased the survival of mice infected with *Staphylococcus aureus*. The incidence and development of certain tumors in male Sprague-Dawley rats were reduced by barley bran with 13% total dietary fiber (McIntosh et al., 1996).

2.3.8 Adverse effects of β-glucan

Earlier barley breeding strategies were mainly targeting the reduction of β-glucan in new varieties. β-Glucan can have serious and detrimental effects on the efficiency of malting and brewing process. High levels of β-glucans in malt reflect incomplete wall breakdown and are associated with diminished mobilization of other storage polymers resulting in lower malt extract values (Bamforth, 1985; Bamforh and Barclay, 1993). Residual β-glucan can also cause filtration difficulties in the brewing process and contribute to certain hazes or precipitates that form in stored beer (Bamforth and Barclay, 1993).

Traditionally barley has not been a preferred grain for poultry feed. This was mainly attributed to the low metabolizable energy in barley compared to wheat and corn. In addition, due to β-glucan, barley feeds increase gastric viscosity, interfere with nutrient availability and cause sticky droppings leading to litter problems. However, the use of commercial enzyme preparations has largely eliminated the deleterious effects of barley β-glucans in poultry feeds (Campbell and Bedford, 1992). Enzyme treatment of barley for poultry feed is now well accepted in many countries. Commercial crude enzyme preparations from fungal (*Trichoderma reesei, Aspergillus niger*) and bacterial

(*Bacillus subtilis*) sources are common. The use of these enzymes is largely determined by economic considerations. Ultimately the development of low β-glucan barley varieties for poultry feeds may be the best strategy. Availability of hull-less barley with low crude fiber content has increased the use of barley as poultry feeds (Bhatty, 1993).

Demonstratable health benefits of β-glucan containing diets have been reported especially for cholesterol lowering and attenuation of post-prandial serum glucose and insulin levels. However, in many of the human clinical studies, it has been reported by many researchers that some of their subjects have experienced some minor side effects after consuming β-glucan containing diets (Judd, 1982; Tappy et al., 1996; Behall et al. 1997, 1998; Pick et al., 1998; Anderson et al., 2002). The most frequently reported symptoms after consumption of these high fiber diets was stomach pains, flatulence, feeling full and bloating and occasional diarrhea. However, these are symptoms normally experienced by individuals going from a low fiber to a high fiber diet. It has been suggested that to minimize these minor discomfort, one should start from a low intake of high fiber foods and gradually increase the amounts consumed (Pick et al., 1998). This will generate the microorganisms required to completely ferment the fibers in the colon.

2.3.9 Enzymatic hydrolysis of β-glucan

The most important enzymes in the depolymerization of β-glucan in the starchy endosperm of germinated barley grain are the (1→3)(1→4)-β-glucan-4-glucanohydrolases (EC 3.2.1.73, common name lichenase). It catalyses the hydrolysis of the (1→4)-β-glucosyl linkages in β-glucans only where the glucosyl residue involved is substituted at the C(O)3 position (Woodward et al., 1983). The (1→3)(1→4)-β-glucanases exhibit an endo-action pattern and releases 3-O-β-cellobiosyl-D-glucans (trisaccharides) and 3-O-β-cellotriosyl-D-glucans (tetrasaccharides). In sound, ungerminated grain β-glucanase activity is very low or absent but after one or two days it rises steeply to a maximum on 5-6 days after the initiation of germination (Bamforth and Barclay, 1993). Cellulases (endo-(1→4)-β-glucanase, EC 3.2.1.4) hydrolyse blocks of adjacent (1-4)-β-linkages in barley β-glucan but cellulase level in germinating seeds are very low. Exo-β-glucanases have also been detected in germinating seeds and can hydrolyse β-glucans to release glucose as the only product (Bamforth and Barclay, 1993). Endo-(1→3)-β-glucanases (EC 3.2.1.39) have also been implicated in the initial degradation of β-glucan. However, there are no compelling evidence that purified (1→3)-β-glucanases can hydrolyse single (1→3)-linkages in β-glucans or that the β-glucans contain contiguous β-(1→3)-linkages that can be hydrolysed by this enzyme. Long sequences of β-(1→3)-links do not occur in barley endosperm walls (Woodward et al., 1983).

The above enzymes can hydrolyse barley β-glucans and therefore improve the poultry feed value and also reduce the detrimental effects during malting and brewing processes. However, hydrolysis of β-glucans destroys the polymeric structure of β-glucans and thereby the viscosity forming properties in aqueous solution. The health benefits of β-glucans have been attributed mainly to its viscousness. The presence of these enzymes in products containing β-glucan may result in the loss of these health benefits. However, lichenase has been used extensively to study the structure of β-glucans and is one of the enzymes used in β-glucan assay test kits. It would be interesting to explore the effect of hydrolysed β-glucan on barley starch hydrolysis by α-amylase.

2.4 EXTRUSION COOKING

2.4.1 Introduction

Extrusion is simply the operation of shaping a plastic or dough-like material by forcing it through a restriction or die. Extrusion cooking was developed for the plastic industry but has been adapted by the food industry. Food extrusion has been practiced for over 70 years. Originally it was used for mixing and forming macaroni and ready-to-eat (RTE) breakfast cereal pellets. However, it is now considered as a high temperature short time cooking process and is the most widely practiced technology in the food industry (Faubion, 1982). It is a cost-effective and highly adaptable energy efficient technology, that has the capability to process relatively dry viscous material, improve textural and flavor characteristic of foods, use unconventional ingredients and control thermal changes of food constituents (Harper, 1989).

2.4.2 Equipment design

Food extruders come in two designs; single screw (SSE) and twin-screw extruders (TSE). The SSE consists of a screw rotating in a closely fitting barrel while the TSE have two parallel screws placed in a figure-eight sectioned barrel. Extruders are equipped with jackets that allow heating and cooling of the barrel. TSE can be categorized further according to the various types of screw configurations, where the key point of differentiation is the relative direction of rotation, counter or co-rotating and the degree of screw intermeshing. The most popular of the TSEs are the corotating intermeshing screws because of their higher capacity (Harper, 1989). Preconditioning of the raw material with steam is required in the SSE and not in the TSE, where it is beneficial due to its low energy cost, and results in bigger processing capacity and improved product quality because of less shear (Demetrakakes, 1998).

The SSEs were first used in the food industry in the 1930s while TSE was not developed for the food industry until early 1980. However, TSEs are rapidly becoming the extruders of choice in the food industry because of its advantages. TSEs can handle a wide range of ingredients including sticky and/or otherwise difficult to convey food ingredients, while SSE can only handle flowing granular materials. This has been made possible because TSEs have a greater conveying angle (about 30° in TSE vs 10° in SSE), improved mixing, better heat transfer, less residence time and self-wiping features (Sevatson and Huber, 2000; Harper, 1989). However, SSE is an economical and effective process in cooking and forming pet foods while TSEs are applied to an increasing range of cooking applications, requiring better control and operating flexibility (e.g., processing of confectionery products). TSE are 1.5-2.5 times more expensive than SSE.

A new generation of SSEs was patented by Wenger manufacturing Co, Sabetha, Kansas in 1998 (Riaz, 2000). They are designed to operate at high shaft speed and small length–to-diameter ratios. Their potential advantages include increased capacity, reduced energy consumption, improved processing of high carbohydrate diets, reduced bulk density and reduced processing and capital cost (Riaz, 2000).

2.4.3 Extrusion process

The process can be divided into three general sections, namely feed/conveying, transition/cooking and metering sections. In the feed section the raw material is conveyed in, mixed and compressed to fill the screw flights and conveyed uniformly into the barrel. The transition section is where the cooking takes place, under increased pressure, temperature and compression. Further increase in pressure, temperature and compression takes place in the metering section. Shear occurs as the compressed product is wiped against the wall of the extruder barrel and fed forward against the back pressure created by the die plate. The flights of the extruder screws convey the food material down the barrel and there is a steady increase in pressure along the screw, which reaches its peak in the final metering section just before the die. The homogenized, thermoplastic fluid material is forced through the die, which can be of various shapes. The pressure drop across the die as the material exits causes puffing to occur when the heated water in the product is rapidly converted to steam (flash evaporation) [Faubion, 1982].

2.4.4 Effects of extrusion on food components

The major ingredients in most extruded food products are cereal flour and starches, however, conversion of soy protein into texturized vegetable protein using the SSE is routinely done. The extrusion process variables such as the screw configuration, speed, shear rate, barrel temperature, die size and shape and the moisture content of feed have significant effect on the extruded product (Mercier and Feillet, 1975; Colonna et al., 1984; Chinnaswamy and Hanna, 1988). Extrusion cooking affects all food constituents such as starch, protein, lipids, vitamins, minerals etc in one way or the other. In this review, only starch and β-glucan as affected by extrusion cooking will be discussed.

2.4.4.1 Effect of extrusion cooking on starch

A major difference between extrusion cooking and other forms of food processing is that starch gelatinization occurs at much lower moisture levels (12-20%, w/w) [Camire, 2000]. However, waxy or high amylopectin corn starch extrusion is dominated by melting, which follows zero order kinetics, instead of gelatinization which is a first order reaction (Qu and Wang, 1994). Starch content remained unaffected by extrusion, however, it causes gelatinization and later macromolecular degradation of starch molecules leading to increased starch solubility (Mercier and Feillet, 1975). Starch solubility as measured by water solubility index increases with extrusion temperature (Mercier and Feillet, 1975; McPherson and Jane, 2000). The reduction in moisture content leads to changes from gelatinized-like to dextrinized-like properties (Gomez and Aguilera, 1984). The authors reported that the relative proportion of dextrinized starch increased from about 10 to 60% (w/w) as extrusion moisture content decreased from 23.7 to 7.6% (w/w). Dextrinization appears to become predominant mechanisms of starch degradation during low-moisture high shear extrusion (Gomez and Aguilera, 1983).

Conflicting results have been reported on the effect of extrusion on starch degradation. Colonna et al. (1984) suggested that the molecular degradation of starch was due to random chain fragmentation of both amylose and amylopectin, while Davidson et al. (1984) suggested that the reduction in molecular size was due to fragmentation of amylopectin caused by mechanical rupture of covalent bonds. Chinnaswamy and Hanna 1990) reported that fragmentation of amylopectin molecules was higher than for the amylose molecules. Degradation of starch did not result in the formation of maltodextrins (Mercier and Feillet, 1975; Govindsamy et al. 1996), but Chiang and Johnson (1977) showed significant increase in fructose, glucose, melibiose, maltotriose and maltotetraose which they attributed to the breakdown of (1→4) glucosidic bonds.

Extrusion cooking can be used to direct molecular degradation in order to manufacture dextrins and glucose. High shear conditions are necessary to maximize conversion of starch to glucose (Camire, 2000). Glucose has been produced using the extrusion of barley starch (Linko et al., 1983). An important consequence of starch degradation is reduced expansion. Expansion is usually measured by comparing the diameter of the extrudate to that of the die hole and is related to the product texture. Highly expanded products may crumble easily due to thin cell walls, while dense products are often hard (Chinnaswamy, 1993).

During extrusion cooking terminal reducing sugars on amylopectin branch fragments may form anhydro links with other carbohydrates through transglycosidation process (Theander and Westerlund, 1987). Such novel compounds are likely to be resistant to digestive enzymes and may decrease the nutritive value of starchy foods due to high amount of RS (Camire, 2000). RS can, however, improve certain health conditions by functioning as a dietary fiber.

Limited studies have been directed at investigating the effect of extrusion cooking in a mixed system such as pearled barley flour. However, some researchers report the formation of RS during extrusion (Unlu and Faller, 1998; Huth et al., 2000) while others have shown that no RS is formed during extrusion (Östergard et al., 1989; Parchure and Kulkarni, 1997). Vasanthan et al. (2002) observed an increase in RS3 in extruded high amylose barley flour and not in low amylose barley flour. Unlu and Faller (1998) reported a negative relationship between formation of RS and screw speed. It is therefore, necessary to further explore the impact of various extrusion conditions on the formation of RS and what modifications in the extrusion of starchy material (e.g., pearled barley flour) are required to achieve a high RS content.

2.4.4.2 Effect of extrusion cooking on β-glucan

Extrusion cooking involves high shear and temperature and varying amounts of moisture and therefore may cause physicochemical and nutritional changes in the barley flour. Östergard et al (1989) reported that dietary fiber content of barley increased with extrusion but the total starch content decreased, while Asp and Bjorck (1989) showed that extrusion cooking caused a shift from insoluble to soluble fiber. The effect of extrusion cooking on the primary structure and water solubility of β-glucans from regular and waxy barley was investigated by Jiang and Vasanthan (2000). They reported that extrusion cooking affected the water solubility and the primary structure of β-glucan in a cultivar-dependent fashion. The macromolecular state of β-glucan was preserved when barley flour was extruded in a SSE at 150 °C/120 rpm through a wide die (5mm) (Huth et al., 2000). These researchers reported that the amount of extracted β-glucan was more influenced by the feed moisture than by the temperature of extrusion. The molecular weight of the extrudates ranged from 80,000-125,000 and depended on extrusion temperature. At moisture content of 22.5% (w/w) the molecular weight of the extrudates were 110,000 at 130 °C, 125,000 at 150 °C and 80,000 at 170 °C. Extrusion cooking therefore causes partial depolymerization of the β-glucan.

It is therefore evident from the above discussion that processing affects the solubility and molecular weight of β-glucans, which may in turn affect the potential health benefits that have been attributed to this soluble fiber. To meet the daily intake requirement to satisfy the FDA health claim, β-glucan has to be incorporated into more foods and at higher proportions. There is a need for further research on the effects of extrusion processing under different conditions on β-glucan, especially in barley, as it has not received much attention in the past compared to oats.

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Chapter 3

THE INFLUENCE OF β-GLUCAN CONCENTRATION ON THE *IN VITRO* HYDROLYSIS OF BARLEY STARCH BY PORCINE PANCREATIC α –AMYLASE

3.1 INTRODUCTION

Starch is the major component of most cereal grains and is the major source of energy for humans. Starch can be hydrolysed by acids or enzymes (i.e amylases) to produce dextrins /sugars. Depending on the degree of hydrolysis a number of products may be produced with varying physicochemical properties. Acid conversions give uniform hydrolysate fragments because of random cleavage of the starch molecules, whereas enzymatic treatments produce different oligosaccharides (Inglett 1987, 1990). The susceptibility of starch granules to degradation by α-amylase depends on their botanical origin, which contributes to variations in starch molecular size/structure and granule ultrastucture (Li et al., 2003), enzyme source (Li et al., 2004), physical conditions of the reaction (i.e starch and enzyme concentrations, temperature, pH etc), the viscosity of the medium and any processing or modification the starch may have undergone (Leach and Schoch, 1961; Planchot et al., 1995; Hoover and Zhou, 2003).

β-Glucans are major components in the cell walls of barley and oat endosperms (Fincher, 1975; Bhatty, 1999). They are linear homopolysaccharides consisting of varying proportion of (1-3) and (1-4) β-D-linked glucopyranosyl residues (Wood. 1984). The β (1-3) linkages which are highly flexible occur in isolation and interrupt the regular cellulose like structure in a random manner (Buliga et al., 1986; Henriksson et al., 1995). This irregularity makes β-glucan water soluble (Woodward et al., 1983; Bohm and

Kulicke, 1999). Molecular weights of 4.4×10^4 to 3.0×10^6 have been reported (Carriere and Inglett, 1999). Bohm and Kulicke (1999) have shown that β-glucan in solution exists in two states: molecularly dispersed with normal viscoelastic flow behavior and as an infinite network structure. Barley β-glucan in freshly prepared aqueous solution has been shown to behave like a viscoelastic liquid without any associated structures being detected over a period of several hours despite its ability to form solid gels (Bohm and Kulicke, 1999).

β-Glucans forms a highly viscous solution in water, which makes them potentially useful as gelling or thickening agents in various food and non-food applications. β-Glucans have generated a lot of interest due to its demonstrated human health benefits. A number of studies in both animals (Degroot et al., 1963; Fisher and Griminger, 1967; Kahlon et al., 1993; Hecker et al., 1998) and humans (Anderson et al., 1984; Wood et al., 1989; McIntosh et al., 1991; Yokoyama et al., 1997; Van der Sluijs et al., 1999) have associated β-glucans in prevention and treatment of coronary health disease and diabetes.

The underlying mechanisms of the human health benefits of β-glucan still remain unclear. Many *in vivo* studies elucidating some of the possible mechanisms have been reported (Jenkins et al., 1978; Wood et al., 1990; Wood, 1993; Yokoyama et al., 1997). However, information is scarce on the effect of β-glucan concentration on *in vitro* starch hydrolysis by α-amylase. Many researchers have attributed the beneficial effects of β-glucans to its water solubility and high viscosity (Keagy et al., 2001; Hallfrisch and Behall, 2000; Wood et al., 1994). The high viscosity imparted by β-glucan to aqueous solutions has been attributed to their molecular asymmetry and high molecular weight (Woodward et al., 1983). Increased gut viscosity may reduce starch hydrolysis and impede the uptake of hydrolytic products through the intestinal wall due to increased unstirred water layer (Edwards et al., 1988; Lund et al., 1989). It has been shown that in an aqueous system, dextrins can form a very viscous solution, in the presence of β-glucan (Inglett, 1991).

Through better understanding of the physical and chemical properties of β-glucan and its interactions with other food components e.g starch and its enzymic hydrolysates, we may be able to increase its use in food and other new novel products. The objective of this study was to investigate the effect of addition of barley β-glucan at different concentration levels (based on the β-glucan content in a low purity β-glucan isolated from CDC-Candle flour) on the kinetics of hydrolysis of barley starch by porcine pancreatic α-amylase. The results obtained may provide an insight into the mechanism by which β-glucan lowers glycemic response.

3.2 MATERIALS AND METHODS

3.2.1 Materials

Barley grains (CDC-Candle cv.) was purchased from Agricore United, Winnipeg, Manitoba. Production of barley flour was carried out at the Centre for Agri-industrial Technology (CAIT), Edmonton, Alberta. Barley grains were dics-pearled in an abrasive disc-dehuller (Baldor Smart Motor, Baldor Electric Co. Ft Smith, ARK., USA) and pin-milled in a Retsch GmbH Ultra Centrifugal mill ZM100 (F. Kurt Retsch GmbH and Co., Hann, Germany) to desired specification (100% pass through a 500 micron screen). Porcine pancreatic α-amylase (EC 3.2.1.1) in 2.9 M NaCl containing 3mM $CaCl_2$ was purchased from Sigma Chemical Co. (St. Lois, MO, USA). Fungal protease (protease 400,000 U/mg) was purchased from Deerland Enzymes (Kennesaw, GA, USA). Lichenase (EC 3.2.1.73; from *Bacillus subtilis*) and β-glucosidase (EC 3.21.21; from *Aspergillus niger*) were purchased from Megazyme International (Wicklow, Ireland). Thermostable α-amylase (from *Bacillus licheniformis*) and amyloglucosidase (EC 3.2.1.3; from *Aspergillus niger*) and the mixed-linkage β-glucan assay kit were purchased from Megazyme International. Termamyl (thermostable α-amylase from *Bacillus licheniformis*) was purchased from Novo Nordisk Biochem, North America Inc. (NC, USA). Termamyl was used during β-glucan isolation process in preference to the Megazyme α-amylase due to its lower cost.

3.2.2 Methods

3.2.2.1 Starch and β-glucan isolation

Starch and β-glucan were isolated from CDC-Candle pearled hull-less waxy barley. The procedure followed (Appendix I) was according to Vasanthan and Temelli (2002).

3.2.2.2 Chemical analysis

Moisture, lipid, protein and starch contents were determined according to standard AACC (2000) procedures. β-Glucan content was determined according to McCleary and Glennie-Holmes (1985) using the β-glucan assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland). The reducing value of the hydrolyzed starch was determined according to the 3,5-dinitrosalicylic acid procedure of Bruner (1964) [Appendix II].

3.2.2.3 *In vitro* α-amylolysis of barley starch in the presence of β-glucan

Starch (5 g) was gelatinized (90-95°C for 15 min) in a beaker containing 200 ml of phosphate buffer (pH 6.9). The starch concentration in the slurry was 2.5%, w/w. Such a low concentration was choosen to avoid excessive viscousness of the reaction mixture since β-glucan itself impart substantial viscosity in aqueous solution. The gelatinized starch slurry was then cooled to 70 °C before adding the appropriate amounts of β-glucan (0, 0.25, 0.5 0.75 and 1.0%, w/v). The amount of β-glucan added was calculated based on the net β-glucan content in the isolated low purity β-glucan. The β-glucan was added slowly while stirring continuously to avoid formation of clumps. When all the β-glucan had been added and solubilized (i.e, form a smooth slurry without ant lumps) the beaker was placed inside a shaking water bath (Lab-line Instuments Inc. Melrose Park, IL.) set at 37 °C and 100 rpm, and left to equilibrate for 1 h. Porcine pancreatic α-amylase (4 units/mg of dry starch) was then added. Aliquots (5 ml) were collected at time intervals of 0-24 h and immediately boiled in a water bath (95-98 °C) for 10 min (to inactivate the enzyme) and then immediately frozen in liquid nitrogen and stored frozen at –18 °C until analysis. The degree of hydrolysis was calculated (Appendix II) by determining the total reducing values in the hydrolysate using the 3, 5-dinitrosalicylic acid procedure of Bruner (1964).

3.2.2.4 β-Glucan hydrolysis by lichenase and determination of the oligosaccharide profile

A study was performed to investigate the effect of native and hydrolysed β-glucan on starch hydrolysis by PAA. For this purpose hydrolysed β-glucan was prepared according to the procedure of Jiang and Vasanthan (2000) with some modifications. Aqueous solution of β-glucan (0.75%, w/v) was prepared using sodium phosphate buffer (20 mM, pH 6.5). The slurry was stirred and incubated in a boiling water bath for 5 min. After cooling to room temperature, lichenase (6000 units/gm) was added and the slurry incubated in a shaking water bath set at 50 °C for 90 min. The β-glucan hydrolysate was then heated in a boiling water bath for 10 min to inactivate the enzyme, cooled and centrifuged at 16,000xg for 10 min.

The study also evaluated the composition of sugar (DP1-DP3)/oligosaccharides (DP4-DP7) released from starch by PAA in the presence of native and lichenase hydrolysed β-glucan. The results presented in Table 3.6 only represents sugar/oligosaccharides profile of hydrolysed starch. The determination of sugar/oligosaccharides profile of lichenase hydrolysed β-glucan was performed in order to ensure that the sugar/oligosaccharides determined and presented in Table 3.4 did not come from hydrolysed β-glucan. The sugar/oligosaccharides profile of PAA hydrolysed starch and lichenase hydrolysed β-glucan was determined as illustrated in section 3.2.2.5

3.2.2.5 Determination of oligosaccharides of the starch hydrolysates

The sugar profiles of the PAA hydrolysates were determined in duplicate by HPLC, using a Jordi Gel DVB Polyamine column (Bellington, MA, USA) of 250 mm length and 4.6 mm diameter. A guard column, All-Guard^{tm} Cartridge System (Alltech Associates, Inc. Deerfield, IL, USA) was inserted prior to the main column. The hydrolysate samples were centrifuged at 16,000 x g for 10 min. A 10μl aliquot of the supernatant was injected into the HPLC system via an auto-sampler (Hewlett Packard, HP 1050 series, Mississauga ON). The sugars were detected using a light scattering detector 500 ELSD (evaporative light-scattering detector) which evaporated the solvent at 125 °C. Two eluents (A:water and B:acetonitrile) were used. Each sample was run for 30 minutes. The elution profile was: 1) 10% A: 90% B at the beginning, 2) 35% A:65% B at 25 min, 3) 0% A:100% B at 26 min and 4) 10% A:90% B at 30 min. A flow rate of 1.0 ml/min was maintained by a Varian 9010 Solvent Delivery System (Sunnyvale, CA, USA). Peak identification and quantification was achieved by external standard solutions of glucose (DP1), maltose (DP2), maltotriose (DP3), maltotetraose (DP4), maltopentaose (DP5), maltohexaose (DP6) and maltoheptaose (DP7). For the sugar/oligosaccharides profile of lichenase hydrolysed β-glucan the above procedure was followed except the flow rate was changed to 1.5 mls/min and total running time was 40 min. The chromatograms (Appendix IV) for lichenase hydrolysed β-glucan/PAA-hydrolysed starch mixtures indicated that α- as well as β-linked saccharides of similar DP were eluted together.

3.2.2.6 Viscosity determination

The viscosity of the hydrolysate (collected at different time intervals) resulting from the action of α-amylase on barley starch in the presence of varying amounts of β-glucan were determined using a PAAR Physica UDS 200 Universal dynamic rheometer (Mebtecknik GmnH Stuttgart, Germany) equipped with a Peltier heat control system. Three measurements were taken at each of the following shear rates; 1.29, 12.9, and 129 s^{-1}, which corresponds to 1, 10, and 100 rpm, respectively. Aliquots (10 g) were collected at time intervals, 0-24 h. The enzyme in the samples was inactivated by boiling for 10 minutes. After cooling to about 40 °C, 7 g was weighed directly into a rheometer measuring cup. All measurements were performed at sample temperature of 37 °C.

3.2.2.7 Statistical analysis

All measurements were carried out in duplicate. Analysis of variance of the results was performed using the General Linear Model (GLM) procedure of SAS Statistical Software version 8 (SAS Institute, 2000). Multiple comparisons of the means were performed by the Tukey's test at $P<0.05$ level.

3.3 RESULTS AND DISCUSSION

3.3.1 Proximate composition

The composition of the raw material used in this study is presented in Table 3.1. The β-glucan content of pearled barley grains (8.4%, db) was higher than the whole grains (6.5%, db). This can be attributed to β-glucan being the major cell wall component of the endosperm (Fincher 1975, Zheng et al., 2000). The isolated low purity β-glucan and starch had 50.7% β-glucan and 92.7% starch, respectively. The residual starch content of isolated low purity β-glucan was 25.5% and the balance was mostly insoluble dietary fiber.

3.3.2 Influence of β-glucan concentration on the kinetics of starch hydrolysis

The influence of β-glucan concentration on the *in vitro* hydrolysis of gelatinized starch by porcine pancreatic α-amylase is presented in Figs 3.1 and 3.2. The extent of hydrolysis decreased with an increase in β-glucan concentration from 0.25 to 0.75%

Table 3.1. Proximate composition (% w/w db) of barley grain (CDC-Candle) and its isolated components.

Component	Starch	Protein	β-Glucan	Lipid
Whole barley grain	58.5±0.7	12.4±0.2	6.5±0.2	2.1±0.1
Pearled barley flour	73.8±0.4	10.2±0.6	8.4±0.1	1.2±0.1
Isolated starch	92.7±2.3	0.03±0.0	0.2±0.01	0.1±0.0
Low purity β-glucan	25.5±0.2	6.4±0.01	50.7±0.3	0.4±0.01

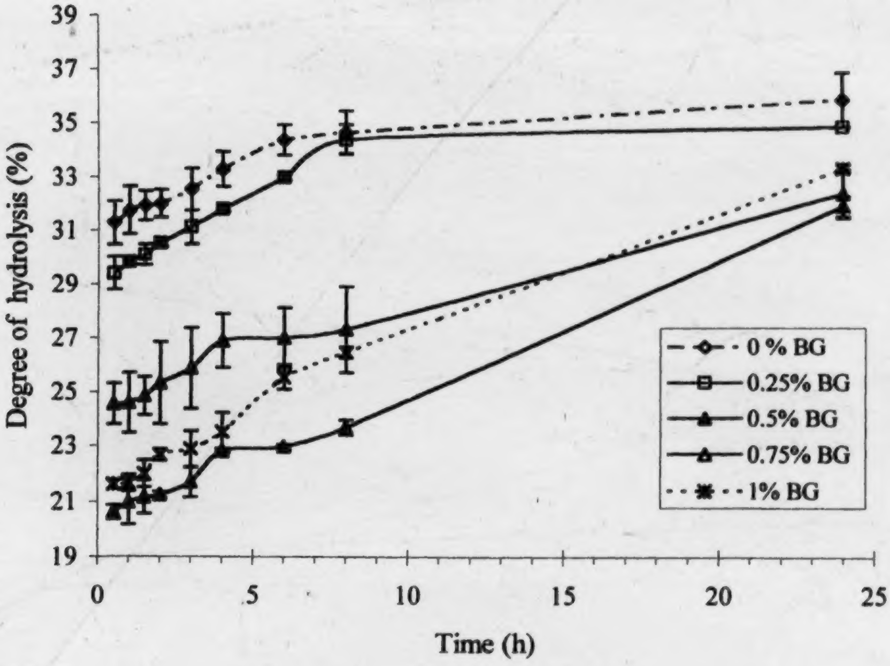

Figure 3.1. Influence of β-glucan concentration (%, w/v) on the hydrolysis of barley starch by porcine pancreatic α-amylase at 37 °C. BG = beta-glucan. Values are means of duplicate determinations, with their standard errors represented by the vertical bars.

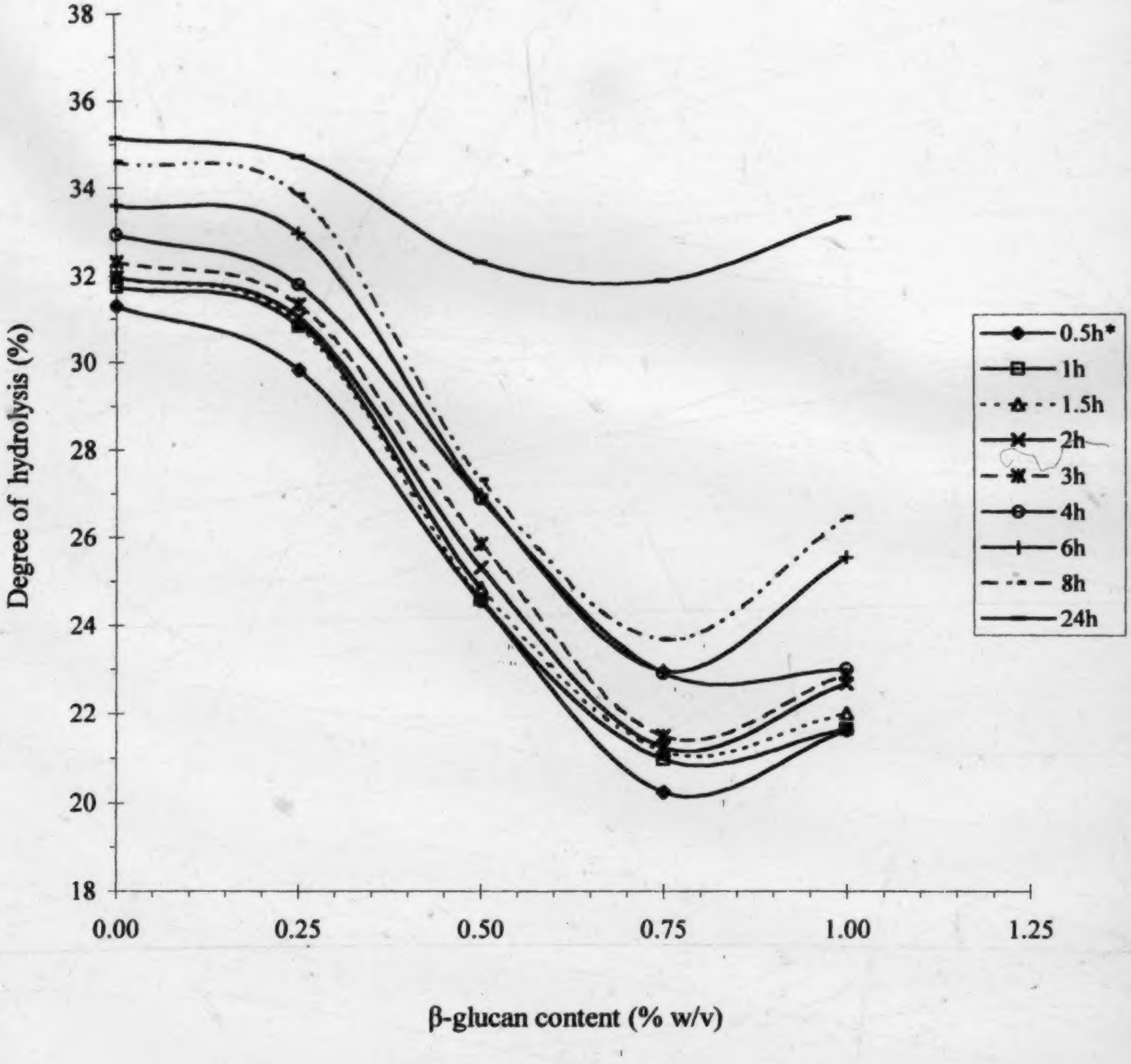

Figure 3.2. Influence of β-glucan concentration on the extent of hydrolysis of barley starch by porcine pancreatic α-amylase at 37 °C. h*=Duration of hydrolysis in hours.

(Fig 3.1). However, at 1.0% β-glucan, the extent of hydrolysis was higher than at 0.75%. In the control sample (0% β-glucan) and at 0.25% β-glucan, the degree of hydrolysis reached a plateau after hydrolysis had been in progress for 6 h and 8 h, respectively. However, at higher β-glucan concentrations, the onset time for the appearance of a plateau (4 h at 0.50 and 0.75% β-glucan, 2 h at 1.0% β-glucan) decreased with an increase in β-glucan concentration. At β-glucan concentrations in the range 0.5-1.0%, the duration of the plateau was much shorter (2 h at 0.50 and 0.75% β-glucan and 1 h at 1.0% β-glucan) than at 1% β-glucan (19 h) and at 0.25% β-glucan (18 h) [Fig 3.1].

The data in Figure 3.1 is presented differently (degree of hydrolysis vs β-glucan content) in Figure 3.2. This is not the conventional way of representing degree of hydrolysis, however, the figure was intended to show the hydrolysis trend as the β-glucan content is increased. It clearly shows how the degree of hydrolysis decreased (0.0-0.75% β-glucan) and thereafter started increasing (0.75-1.0% β-glucan). The initial rates (0.5-2 h) of hydrolysis are presented in Table 3.2. There was no significant ($P<0.05$) change in the initial rate at 0.25 and 1.0% β-glucan. However, the initial rate decreased by 20.0 and 37.5% at 0.50 and 0.75% β-glucan, respectively.

It is necessary at this stage to give a brief description of the mechanism of α-amylase action on starch hydrolysis, which will enable a subsequent discussion of the influence of β-glucan concentration on the kinetics of hydrolysis. The active site of PAA has been shown to consist of an array of tandem subsites, with each subsite complimentary to interacting with a single monomer residue of the substrate polymeric chain (Robyt, 1984). Five subsites have been proposed to account for the enzymatic properties of PAA (Robyt, 1984). The scission occurs between subsites 2 and 3.

Table 3.2. The influence of β-glucan concentration on the initial rate[1] of α-amylolysis of gelatinized barley starch.

β-glucan reaction mixtures (% w/v)	Initial rate of amylolysis (reducing value/hour)
0.00	0.64^{a}
0.25	0.61^{ab}
0.50	0.51^{c}
0.75	0.40^{d}
1.00	0.60^{ab}

[1] The initial rate of reaction was calculated from the regression passing through 0.5h - 2h. The rate was expressed as dRV/dt, where dRV is change in reducing value and dt is the change in time (h).

[a-d] Means within the column with different letters are significantly different ($P<0.05$).

The glucose residue at the reducing end occupies the lowest numbered subsite. α-amylase has been shown to form two types of enzyme-substrate (ES) complexes (Kuriki and Imanaka, 1999). In the so called productive ones (ES_{pr}) the substrate chain overlaps the catalytic site and then can be hydrolyzed. Whereas, in the non-productive complexes (ES_{np}) the chain is bound only at one side of the catalytic center and thus the substrate remain unhydrolysed. Thus with short chain substrates (maltose, maltotriose) several complexes of each type can be formed and a consequence of this is that the non-productive complexes are inhibitory (competitive inhibition) to α-amylase action. Planchot et al (1997) have shown that the action of α-amylase on starch consists of the following three steps: 1) diffusion of the enzyme towards the substrate, 2) adsorption of the enzyme on the substrate surface, and 3) catalytic action. In the present study the mobility of the enzyme (α-amylase) and the substrate may have been influenced by the porosity of the β-glucan network.

The decrease in the extent of hydrolysis on the addition of β-glucan in the concentration range 0.25-1.0% to gelatinized starch (Figs. 3.1 and 3.2) could be attributed to increased viscosity of the reaction medium due to the interplay of the following factors: 1) molecular asymmetry of β-glucan, 2) high molar mass of β-glucan, 3) formation of a network (i.e fringed micelle) structure between β-glucan chains, 4) interaction between β-glucan and gelatinized starch and, 5) interaction between β-glucan and the products of α-amylolysis. An increase in viscosity could decrease the rate of diffusion of α-amylase to gelatinized starch, thereby, reducing the extent of hydrolysis.

Although, this explains the decrease in hydrolysis with increase in β-glucan concentration in the range of 0.25-0.75%, it does not explain, the reversal in hydrolysis at 1.0% β-glucan (Figs 3.1 and 3.2). We therefore, postulate that in the concentration range of 0.25-0.75%, the added β-glucan interacts with gelatinized starch either *via* hydrogen bonding or by being physically adsorbed on to the starch surface. Both of the above mechanisms could decrease the accessibility of α (1→4) glycosidic linkages of gelatinized starch to the active site of α-amylase, thereby, decreasing hydrolysis. However, at 1.0% β-glucan, the concentration may be optimal for micelle formation between β-glucan molecules (Varum et al., 1992; Grimm et al., 1995; Zhang et al., 1997). Thus, if the rate and extent of β-glucan micelle formation exceeds the rate and extent of interaction between β-glucan and gelatinized starch molecules, then more gelatinized starch would become available for interaction with α-amylase at 1.0% β-glucan. This would then explain the reversal at 1.0% β-glucan.

The appearance of a plateau at 0 and 0.25% β-glucan suggests inhibition of α-amylase activity by the products of α-amylolysis (maltose, maltotriose and maltotetraose). The shortening of the duration of plateau with increase in β-glucan concentration (Fig. 3.1) can be attributed to interaction (*via* hydrogen bonding) between β-glucan and the products of α-amylolysis, and/or to the slower rate of diffusion of the products of α-amylolysis to the catalytic center of α-amylase (this seems plausible, since the viscosity of the reaction medium increases with increase in β-glucan concentration) [Table 3.4].

3.3.3 The Influence of lichenase hydrolysed β-glucan on the α-amylolysis of gelatinized starch

The influence of native and lichenase hydrolysed β-glucan (0.75%, w/v) on the α-amylase hydrolysis of gelatinized barley starch solution (2.5%, w/v) is presented in Fig 3.3. The extent of α-amylolysis of gelatinized starch was higher in the presence of lichenase hydrolysed β-glucan. This suggests that the effectiveness of β-glucan in reducing the extent of hydrolysis of gelatinized starch by α-amylase is dependent to a great extent on its chain length. A decrease in β-glucan chain length by lichenase hydrolysis would decrease its molar mass, thereby reducing solution viscosity. Consequently, the rate of diffusion of α-amylase towards the starch molecular surface and hence the extent of starch hydrolysis would be higher in the presence of lichenase hydrolysed β-glucan (Fig 3.3). This explanation seems plausible, since the difference in solution viscosity in the presence of intact (4100 mPa s at a shear rate of 1.29 s^{-1}) and lichenase hydrolysed (135 mPa s at a shear rate of 1.29 s^{-1}) β-glucan was significant (Table 3.3). The increase in hydrolysis in the presence of hydrolysed β-glucan is of importance in increasing the feed value of barley based animal feeds (Klopfenstein, 1988). However, in human food, the nutritional health benefits that have been attributed to β-glucans especially the moderation of post-prandial glucose and insulin levels in diabetics and cholesterol levels in hypercholesterolemia individuals, would be lost with such a treatment to β-glucan with lichenase, since these health benefits have been attributed mainly to the high viscosity of the β-glucan in solutions (Wood et al., 1994).

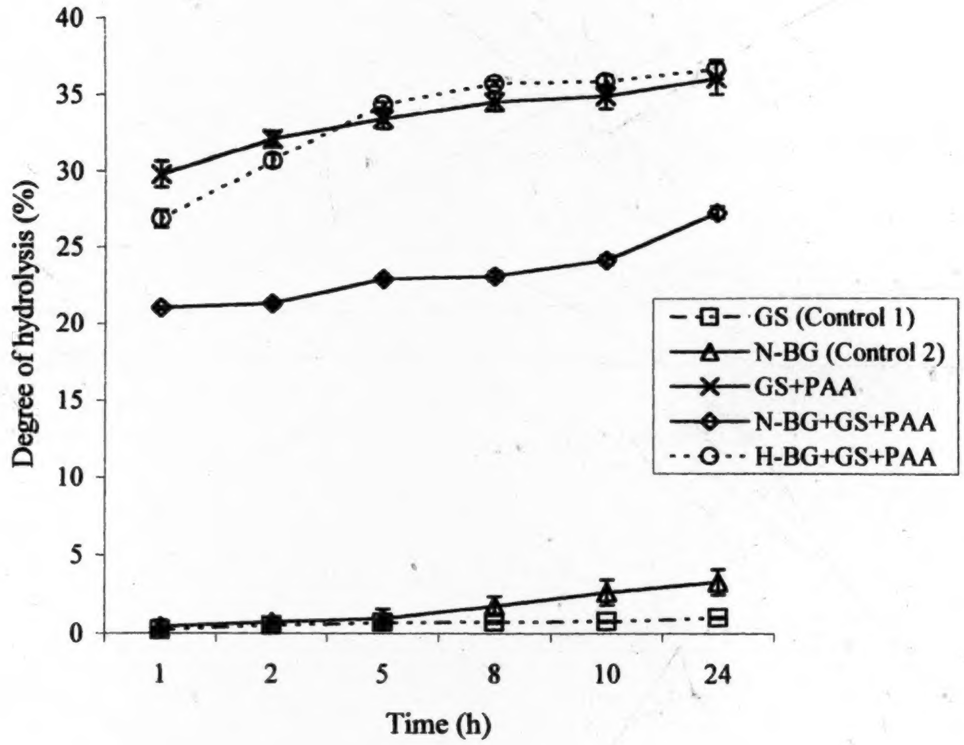

Figure 3.3. The effect of native (N-BG, 0.75% w/v) and lichenase hydrolysed (H-BG, 0.75% w/v) β-glucan on the hydrolysis of gelatinized starch (GS, 2.5% w/v) by porcine pancreatic α-amylase (PAA). Values are mean of duplicate determinations with their standard errors represented by the vertical bars.

Table 3.3. The viscosity[1] of native β-glucan (0.75%, w/w) and lichenase hydrolysed β-glucan (0.75%, w/w) at shear rate of 1.29 s^{-1}.

Material	Viscosity (mPa s)						
	Incubation period at 37 °C (h)						
	0	1	2	4	6	8	24
Native β-glucan	4300±98	4030±29	4200±55	4000±48	3950±83	3850±90	3550±84
Hydrolysed β-glucan	135±7	110±5	129±4	138±7	102±3	133±7	nd[2]

[1] Duplicate determinations ± standard deviation

[2] nd - Not determined

3.3.4 Rheological properties

Effect of shaking in a water bath at 37 °C:

The viscosity (determined at a shear rate of $1.29s^{-1}$) of gelatinized starch (2.5%, w/v), β-glucan (0.75%, w/v), β-glucan (0.75%, w/v) plus gelatinized starch (2.5%, w/v) blend and β-glucan plus PAA at various time intervals while being mixed in a shaking water bath are presented in Fig 3.4. The viscosity of gelatinized starch was below 500 mPa s throughout the time course (24 h) of hydrolysis. Beta-glucan showed a high initial viscosity (4500 mPa s), which decreased gradually, reaching a value of 3500 mPa s at the end of 24 h of mixing. Several researchers have shown that solutions of β-glucan display shear-thinning behavior over shear rates of 1-1500 s^{-1} (Wood, 1984; Autio et al., 1987; Wilkstrom et al., 1994; Dawkins and Nnanna, 1995; Doublier and Wood, 1995; Zhang et al., 1998; Carriere and Inglett, 1999; Skendi et al., 2003; Lazaridou et al., 2003).

The viscosity of the β-glucan-gelatinized starch blend (prepared by gelatinizing starch first and then gradual solubilizing of the β-glucan into the same solution) was substantially lower than that of β-glucan, but higher than that of gelatinized starch. This could be attributed to the presence of starch molecules in between β-glucan molecules, interrupting the β-glucan micelle formation that is important for viscosity development (Varum et al., 1992; Grimm et al., 1995; Zhang et al., 1997). Furthermore, unlike the viscosity profiles of β-glucan or gelatinized starch the viscosity of the β-glucan-gelatinized starch blend increased gradually during the first 16h and then stabilized thereafter. The addition PAA to β-glucan solution resulted in a rapid increase in viscosity (Fig. 3.4). This could be attributed to interaction between β-glucan and dextrins (resulting from the action of PAA on the residual starch associated with β-glucan).

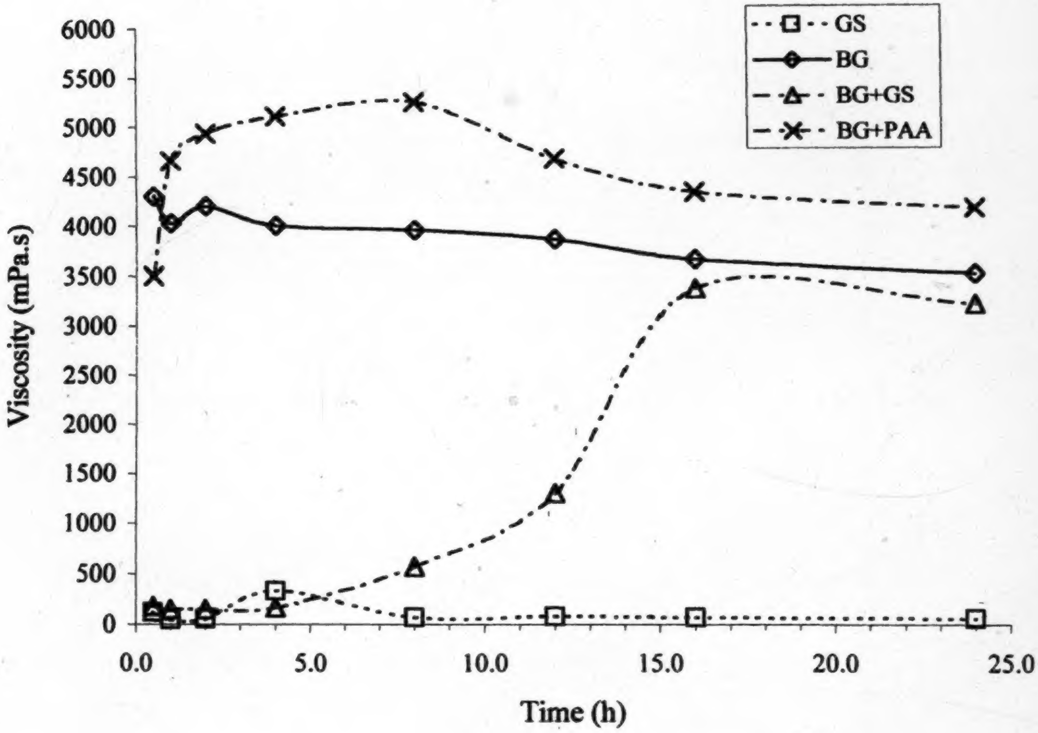

Figure 3.4. Viscosity (at a shear rate of 1.29 s^{-1}) profiles of β-glucan (BG, 0.75% w/v), gelatinized starch (GS, 2.5% w/v), BG-GS blend (0.75% and 2.5% w/v, respectively) and BG (0.75% w/v) treated with α-amylase (4U/mg).

The above difference in viscosity between β-glucan and PAA treated β-glucan was maintained throughout the time course of the viscosity measurement. However, the magnitude of this difference was highest at ~ 8 h and was less pronounced thereafter (Fig. 3.4). This suggests that the network structure formed by β-glucan (in the presence of dextrins) at a concentration of 0.75%, is unstable to prolonged shaking (shear), that the extent of the breakdown of the micelle structure negates the viscosity gain (shear-thickening) resulting from β-glucan-dextrin interaction.

The influence of β-glucan concentration on the viscosity of β-glucan-gelatinized starch blend that is being hydrolysed by PAA:

Viscosity (a rheological parameter) is a measure of the resistance to flow of molecules in solution. Changes in rheological properties of a polymer reveal changes in its molecular structure, conformation and concentration. The influence of β-glucan concentration on the viscosity of PAA hydrolysed gelatinized starch at different time intervals of hydrolysis are presented in Table 3.4. The results show that in solutions containing gelatinized starch (2.5%, w/v) with added β-glucan (0.25-1.00%), the viscosity increased rapidly during the first 5 min after addition of α-amylase. This increase may be attributed to two factors: (a) Hydrolysis of starch that was originally present in between β-glucan molecules. This would have enabled β-glucan molecules to form micelle structures, and (b) β-glucan-dextrin interactions. This explanation seems plausible since the extent of viscosity increase became less pronounced with increase in shear rate (1.29-129 mPa s). In the absence of β-glucan (0%, w/v), gelatinized starch was hydrolysed at a faster rate by PAA. This was indicated by the rapid decrease in viscosity

Table 3.4. The viscosity profiles[1] of gelatinized starch (2.5%, w/v) and β-glucan (0.25-1.00%, w/v)-gelatinized starch (2.5%, w/v) blend during (0 min-24 h) α-amylolysis.

β-Glucan content (%)	Shear rate (1/s)	Viscosity (mPa s)							
		Duration of hydrolysis							
		0h	5min	1h	2h	4h	6h	8h	24h
0.00	1.29	24 ± 1.1^{a}	122 ± 4.2^{a}	2.75 ± 0.1^{a}	-[2]	-	-	-	-
	12.9	17 ± 0.7^{a}	42 ± 0.4^{a}	0.98 ± 0.2^{a}	-	-	-	-	-
	129	13 ± 0.5^{b}	18 ± 0.1^{a}	1.06 ± 0.0^{c}	-	-	-	-	-
0.25	1.29	39.1 ± 0.3^{a}	125 ± 10.6^{a}	79.1 ± 0.1^{a}	71.4 ± 0.1^{a}	65.3 ± 0.3^{a}	42.6 ± 1.8^{a}	32.7 ± 0.1^{a}	IS[3]
	12.9	20.5 ± 1.0^{a}	60.0 ± 4.2^{a}	58.1 ± 0.2^{a}	54.5 ± 0.1^{a}	52.5 ± 0.1^{a}	38.3 ± 0.2^{a}	31.4 ± 0.1^{a}	"
	129	19.9 ± 1.1^{c}	35.9 ± 1.8^{a}	27.9 ± 0.2^{b}	27.5 ± 0.0^{b}	27.6 ± 0.1^{b}	23.7 ± 0.0^{bc}	21.4 ± 0.0^{c}	"
0.50	1.29	109 ± 7.1^{c}	214 ± 1.4^{c}	1325 ± 35.4^{b}	1345 ± 7.1^{b}	1560 ± 14.1^{a}	1495 ± 7.07^{a}	1220 ± 14.1^{b}	IS
	12.9	67 ± 6.0^{c}	79.6 ± 4.5^{c}	524 ± 2.8^{b}	549 ± 0.0^{b}	602 ± 2.8^{a}	574 ± 2.8^{ab}	518 ± 1.4^{b}	"
	129	41.8 ± 2.4^{e}	68.2 ± 3.6^{d}	139 ± 0.7^{c}	147 ± 0.0^{b}	151 ± 0.7^{a}	148 ± 0.7^{ab}	141 ± 0.5^{bc}	"
0.75	1.29	168 ± 17.1^{e}	409 ± 35^{e}	6595 ± 219^{b}	6810 ± 184^{b}	6820 ± 212^{b}	7265 ± 134^{a}	6090 ± 184^{c}	1305 ± 7.8^{d}
	12.9	99.8 ± 5.9^{e}	132.5 ± 18^{e}	1860 ± 28.3^{c}	1930 ± 28.2^{b}	1915 ± 21.2^{b}	2050 ± 28.3^{a}	1810 ± 28.0^{c}	1002 ± 0.7^{d}
	129	95.8 ± 3.4^{d}	93.7 ± 4.3^{d}	354 ± 0.7^{c}	364 ± 1.41^{b}	363 ± 2.8^{b}	389 ± 1.4^{a}	355 ± 4.95^{c}	77.4 ± 0.4^{e}
1.00	1.29	368 ± 13.5^{f}	587 ± 14.2^{f}	19300 ± 78^{a}	18400 ± 120^{b}	18700 ± 707^{b}	18100 ± 700^{bc}	16950 ± 212^{d}	4805 ± 6.4^{e}
	12.9	215 ± 27.6^{g}	225 ± 7.1^{g}	4315 ± 35.4^{a}	4200 ± 56.7^{b}	4035 ± 91.9^{c}	4015 ± 49.5^{d}	3950 ± 42.4^{e}	3170 ± 1.4^{f}
	129	135 ± 4.2^{g}	107 ± 0.8^{h}	681.5 ± 2.1^{a}	674 ± 2.8^{b}	636 ± 2.1^{e}	658 ± 2.1^{c}	639 ± 0.7^{d}	263 ± 0.7^{f}

[1] Values are means of duplicate determinations±SD. Means in the same row not sharing a superscript letter are significantly different ($p<0.05$).

[2] Values too low to be detected

[3] Insufficient sample

from 122 mPa s to 2.75 mPa s within 55 min (Table 3.4). However, in the presence of 0.25% β-glucan, the viscosity decrease was more gradual, reaching a value of 32.7 mPa s after 8 h of hydrolysis. At β-glucan concentrations of 0.50, 0.75 and 1.00%, the viscosity increased rapidly during the first hour of hydrolysis, and thereafter the increase was more gradual until 6h hydrolysis. The extent of this increase increased with β-glucan concentration (Table 3.4). At 0.50, 0.75 and 1.00% β-glucan, viscosity began to decrease when hydrolysis was continued beyond 6h. The results suggest that maximum viscosity is influenced by β-glucan concentration and duration of hydrolysis.

Perhaps the slower rate of hydrolysis at β-glucan concentrations of 0.25, 0.5 and 0.75%, increased the duration of hydrolysis that is required to generate starch chains that are optimal in size for viscosity development. At 0.25% β-glucan, the maximum viscosity was attained at 5 min. Whereas, at 0.50 and 0.75% β-glucan, maximum viscosity was attained at 4h and 6h of hydrolysis, respectively. However, at 1% β-glucan the maximum viscosity was reached at 1h of hydrolysis, perhaps due to increased rate of hydrolysis (Table 3.2). The increase in viscosity reflects interaction between β-glucan and the initial products of α-amylolysis (maltodextrins). The decrease in viscosity beyond 6h hydrolysis, can be attributed to the presence of smaller molecular size starch/sugar fragments, that may have resulted from the further action of PAA on maltodextrins. The chain length of these sugar fragments are probably too small (non-optimal in size) to effectively interact with the β-glucan chains.

We postulate that at concentrations exceeding 0.25%, β-glucans form a micelle network structure, which physically entraps maltodextrins, within and between the micelles, as illustrated in Fig 3.5. This would enable maltodextrins to interact strongly

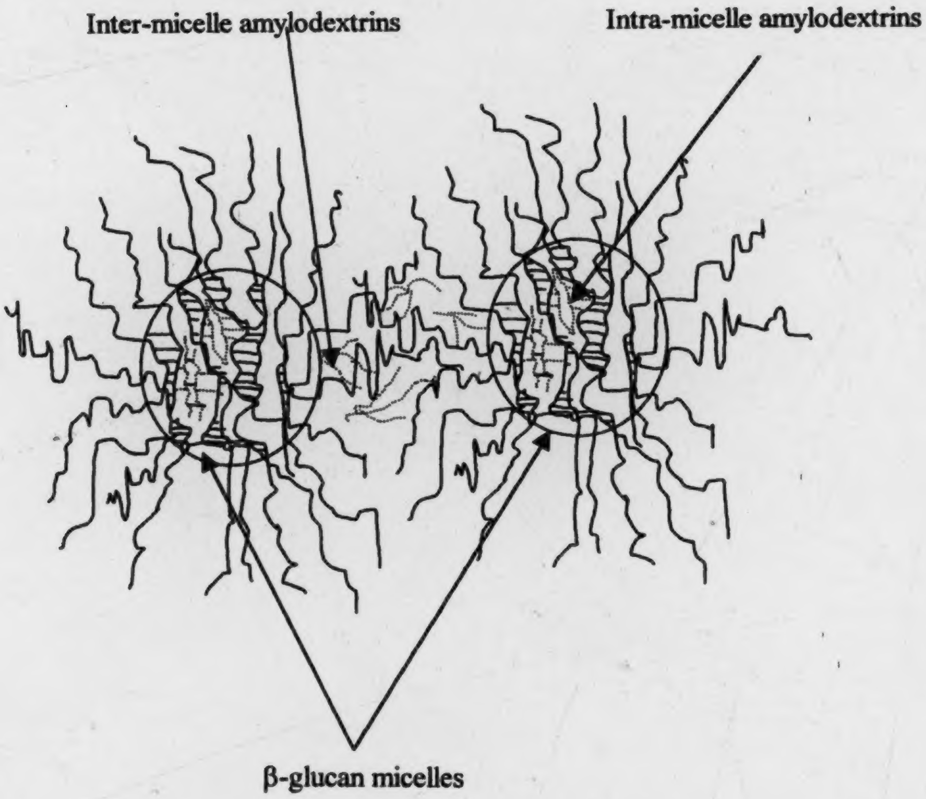
Inter-micelle amylodextrins
Intra-micelle amylodextrins
β-glucan micelles

Figure 3.5. Fringed micelles due to aggregation of β-glucans strands interconnected with amylodextrins. Solid line = β-glucan strands, dotted lines = amylodextrins.

(*via* hydrogen bonding) with the hydroxyl groups of β-glucan, thus hindering their further hydrolysis into smaller molecular weight sugar fragments. This seems plausible, since the decrease in viscosity occurs after only 1h hydrolysis at 0.25% β-glucan, but occurs much later (6h) at the higher concentrations (0.5-1.00%) [Table 3.4]. These results suggest that maximum viscosity development between β-glucans and PAA hydrolytic products (maltodextrins) require an optimum molecular size of dextrin. The viscosity profiles of gelatinized starch and β-glucan-gelatinized starch blends showed the characterisctic shear-thinning at shear rates (1.29–129 s^{-1}) [Table 3.4].

The shear-thinning behavior at each time of hydrolysis (0-24 h) increased with increase in β-glucan concentration (Table 3.4). This is typical of most polysaccharide solutions (Doublier and Wood, 1995). This pseudoplasticity, observed with increasing shear rate appears to be important in contributing to good sensory qualities (e.g mouth feel and flavour) to foods and is important in pourability of suspensions and emulsions (Dawkins and Nnanna, 1995). However, Carriere and Inglett (1999) reported observing two different shear rate regions (30 and 100 s^{-1}) of shear-thickening in oatrims (oat β-glucans/amylodextrin blends) at 24 % β-glucans by weight. They also observed a shear-thickening region at 10 % β-glucans but did not observe this shear thickening at the 5 % level. They postulated the shear thickening behavior is influenced by β-glucan concentration and interactions between β-glucans and amylodextrins.

The viscosity (at different shear rates) determined at different time intervals during amylolysis of gelatinized starch (2.5%, w/v) mixed with lichenase hydrolysed β-glucan (0.75%, w/v) is presented in Table 3.5. The viscosity of this blend was not only lower than that of the native unhydrolysed β-glucan (0.75%, w/v)-gelatinized starch

Table 3.5. The influence of shear rate on the viscosity profiles of lichenase hydrolysed β-glucan (0.75% w/v)-gelatinized starch (2.5% w/v) blend during α-amylolysis.

Shear Rate (1/s)	Viscosity (mPa s)						
	Duration of hydrolysis						
	0h	1h	2h	4h	8h	10h	24h
1.29	34±4[1]	32±4	31±3	30±3	29±4	27±5	27±6
12.90	13±1	10±1	10±1	11±1	10±1	9±1	9±1
129.00	7±0	5±0	4±0	5±0	4±0	4±0	4±0

[1] Mean values of duplicate analysis.

(2.5%, w/v)-PAA blend (Table 3.4), but also remained fairly stable throughout the time course of hydrolysis. This suggests lichenase hydrolysed β-glucan contains mainly low molecular weight sugar fragments (Jiang and Vasanthan 2000) and consequently, is unable to interact with maltodextrins released from the action of PAA on gelatinized starch. Thus, only intact β-glucan can interact effectively with maltodextrins. Doublier and Wood (1995) showed that partially acid hydrolysed oat β-glucan exhibits a high viscosity and the typical shear thinning behaviour of pseudoplastic materials. But the viscosity was lower than that of the unhydrolysed β-glucan especially at a relatively high shear rate of 100 s^{-1}. Their results indicated that partial hydrolysis of β-glucan does not destroy completely its thickening ability in solution. However, the enzymic hydrolysis conditions used in the present study showed that lichenase hydrolysed β-glucan was converted quantitatively mainly to DP3 and DP4 as shown by the sugar/oligosaccharides HPLC profile in Table 3.6.

3.3.5 Sugar profile as determined by HPLC

Analysis of the fine structure of β-glucan can be achieved by enzymic degradation with lichenase. The enzyme lichenase, (1→3)(1→4)-β-D-glucan-4-glucanohydrolase, specifically cleaves the (1→4) glycosidic bonds of 3-substituted glucose residue of β-glucan (Woodward et al., 1983). The major products are 3-O-β-cellobiosyl-D-glucose (a trimer, DP3) and 3-O-β-cellotriosyl-D-glucose (a tetramer, DP4). However, cellodextrin-like oligosaccharides of higher DP are produced from regions of the polymer containing more than three consecutive (1→4)-linked glucose units (Doublier and Wood, 1995).

Also, there is evidence that minor amounts of sequences with consecutive (1→4) linkage longer than the 4 units and up to 14 glucosyl units do exists (Cui and Wood, 2000). The sugar profiles (DP1-DP7) of lichenase hydrolysed β-glucan (0.75%, w/v) plus gelatinized starch (2.5%) blend is reported in Table 3.6. The major hydrolytic products were DP3 and DP4, however, some DP2, DP5, DP6 and DP7 were also detected but no DP1.

Oligomers higher than DP7 in the HPLC chromatogram were observed, however, due to unavailability of authentic oligosaccharides standards we could not quantify these oligomers. The results are consistent with reports in literature. It has been shown by a number of researchers (Doublier and Wood, 1995; Cui and Wood, 2000; Lazaridou et al., 2003; Skendi et al., 2003) that the main lichenase hydrolytic products (tri and tetramer sugars) from oats and barley make up 90-95% of the total oligomers released.

In the presence of PAA, native β-glucan-gelatinized starch blend showed an increasing amount of sugars as compared to control 2. DP2 was the major product and it increased with duration of hydrolysis. The amount of DP3 was also high initially but it decreased with the extent of PAA hydrolysis. Some DP5-DP7 was also detected after four hours of hydrolysis. Generally DP1 and DP5 increased with PAA hydrolysis while DP6 and DP7 was fairly constant. DP4 was not detected in the native β-glucan-gelatinized starch blend in the presence of PAA.

There was an increase in all the sugars (DP1-7) when hydrolysed β-glucan-gelatinized starch blend in the presence of PAA was analysed (Table 3.6). The major components were DP2 and DP3. This was expected as the major hydrolytic products of lichenase hydrolysed β-glucan is DP3, while for PAA it is DP2. Generally in this blend/slurry DP1, DP2 and DP5 increased with increasing hydrolysis time while DP3

Table 3.6. HPLC sugar[1] profile analysis of native and lichenase hydrolysed β-glucan and starch slurry in the presence or absence of porcine pancreatic α-amylase.

Duration of Hydrolysis (h)	Amount (mg/ml) of sugar						
	DP1*	DP2	DP3	DP4	DP5	DP6	DP7
Lichenase hydrolysed β-glucan (0.75% w/v, Control 1)							
	0.00±0.00	0.39±.02	32.42±1.6	13.82±1.13	1.11±0.11	0.79±0.00	0.97±0.15
β-Glucan (0.75% w/v) and starch (2.5% w/v, Control 2)							
0	0.00±0.00	0.00±0.00	0.23±0.02	0.33±0.07	0.24±0.09	0.00±0.00	0.00±0.00
Native β-glucan (0.75% w/v) and starch (2.5% w/v) plus alpha-amylase (4U/mg)							
0	0.00±0.00	0.00±0.00	0.15±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1	0.23±0.00	3.72±0.18	2.89±0.09	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	0.16±0.01	3.57±0.04	3.24±0.03	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
4	0.46±0.03	5.16±0.02	2.73±0.03	0.00±0.00	0.24±0.02	0.74±0.08	0.55±0.12
8	0.72±0.01	5.51±0.27	1.72±0.00	0.00±0.00	0.31±0.06	0.75±0.14	0.53±0.15
10	1.36±0.04	11.78±1.67	0.46±0.00	0.00±0.00	0.45±0.05	0.90±0.17	0.50±0.13
24	2.33±0.00	17.81±2.21	0.24±0.01	0.23±0.01	0.71±0.05	0.78±0.08	0.39±0.02
Hydrolysed β-glucan (0.75% w/v) and starch (2.5% w/v) plus alpha-amylase (4U/mg)							
0	0.00±0.0	14.54±1.44	6.66±0.83	3.46±0.27	0.51±0.07	0.00±0.00	0.00±0.0
1	0.58±0.02	23.21±0.19	31.29±0.63	3.99±0.17	1.69±0.22	2.73±0.03	2.42±0.21
2	1.23±0.01	28.03±0.01	31.17±0.69	4.06±0.64	1.99±0.13	3.12±0.11	2.78±0.10
4	2.70±0.05	33.10±0.91	27.32±1.13	3.97±0.49	2.27±0.14	3.31±0.27	2.24±0.19
8	3.79±0.17	35.05±0.08	23.58±0.30	4.09±0.59	2.53±0.26	3.60±0.34	2.78±0.15
10	4.79±0.07	36.34±0.90	21.35±0.36	4.00±0.44	2.47±0.13	3.43±0.24	2.40±0.18
24	0.70±0.10	40.30±1.71	16.80±1.32	5.03±0.88	2.90±0.24	3.41±0.42	2.48±0.48

[1] DP1* - Glucose, DP2 - Maltose, DP3 - Maltotriose, DP4 - Maltotetraose, DP5 - Maltopentaose, DP6 - Maltohexaose, and DP7 – Maltoheptaose

decreased. DP4, DP6 and DP7 remained fairly constant after the first hour of hydrolysis. There was an increase in all the sugars (DP1-7) when hydrolysed β-glucan-gelatinized starch blend in the presence of PAA was analysed (Table 3.6). The major components were DP2 and DP3. This was expected as the major hydrolytic products of lichenase hydrolysed β-glucan is DP3, while for PAA it is DP2. Generally in this blend/slurry DP1, DP2 and DP5 increased with increasing hydrolysis time while DP3 decreased. DP4, DP6 and DP7 remained fairly constant after the first hour of hydrolysis.

The amount of sugars released during PAA hydrolysis of gelatinized starch in the presence of native β-glucan was far less than in the presence of lichenase hydrolysed β-glucan. In the presence of native β-glucan, the amounts of DP2 and DP3 ranged from 0-17.8 and 0.15-3.25 mg/ml, respectively. While in the presence of lichenase hydrolysed β-glucan it ranged from 14.5-40.3 and 6.6-31.3 mg/ml for DP2 and DP3, respectively. The HPLC sugar profile results are consistent with the increase in total reducing value (reported and discussed earlier in Figures 3.1 and 3.2).

3.4 CONCLUSIONS

The effect of β-glucan content on starch hydrolysis by porcine pancreatic α-amylase (PAA) was investigated. Starch hydrolysis decreased with increase in β-glucan content from 0–0.75% but increased thereafter. Hydrolysis of β-glucan with lichenase, prior to PAA addition, did not decrease starch hydrolysis. It was evident from the study that viscosity was the key factor responsible for the observed decrease in starch hydrolysis. Viscosity measurements showed that β-glucan was able to interact with hydrolytic products (dextrins) resulting from action of α-amylase on gelatinized starch. The magnitude of these interactions was influenced by dextrin chain length. However, β-glucan-dextrin interaction was not observed with lichenase hydrolysed β-glucan. The results of this study have nutritional and functional importance in the use of barley β-glucan in food and non-food applications.

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Chapter 4

THE INFLUENCE OF α-AMYLASE HYDROLYSED BARLEY STARCH FRACTIONS ON THE VISCOSITY OF LOW AND HIGH PURITY BARLEY β-GLUCAN CONCENTRATES

4.1 INTRODUCTION

Barley β-glucan is a water soluble dietary fiber that can form highly viscous aqueous solutions at concentrations as low as 0.5% (w/v) (Autio et al., 1987; Doublier and Wood, 1995; Bohm and Kulicke, 1999; Burkus and Temelli, 1999; Cui and Wood, 200; Skendi et al., 2003; Lazaridou et al., 2003; Vaikousi et al., 2004). Research on barley β-glucan has demonstrated its multiple human health benefits (Anderson et al., 1984; Wood et al., 1994; Braaten et al., 1994; Yokoyama et al., 1997; Bourdon et al., 1999; Hallfrisch and Behall, 2000; Mälkki and Virtanen, 2001; Keagy et al., 2001). Thus, the industrial demand for this natural cereal based compound is fast growing. Functional food products containing β-glucan are now being commercially introduced to the market (Wood, 1986; Inglett, 1990; Pszczola, 1996; Temelli, 2001; Morin, 2001; Burkus and Temelli, 2001). Since starch is one of the major components of foods, understanding the mechanism of interaction of β-glucan with native starch and its hydrolytic products and its implication on rheological properties, is highly important in order to achieve a product with a high sensory appeal.

Studies focusing on the rheological response arising from the interactions between β-glucan and other biopolymers, especially the interaction of β-glucan with starch amylolytic products are limited. The effect of starch on the viscosity of barley β-glucan gum was investigated by Burkus and Temelli (1998). The authors reported that the viscosity of β-glucan (0.5% w/w) in the presence of starch (0.5% w/w) increased by about 2.5-fold over β-glucan. However, the gum mixture separated into two layers following storage at 4 °C. They postulated that the hydrophobicity of the interior of the hydrogen-bonded regions of amylose and β-glucan may, in part, be responsible for such destabilization. Prehydration of the starch solution slowed down the separation of the phases. Carriere and Inglett (1999) investigated the rheological properties of several Oatrim® (β-glucan/amylodextrin) blends. Shear–thickening regions observed in their study were dependent upon the temperature of the suspension and the concentration of β-glucan in the blend. Grimm et al (1995) used rheological methods to investigate the solution properties (behaviour of molecular aggregates) of β-glucan isolated from barley grains and beer. In this study, maltose solutions at different concentrations (2-10%, w/v) were used to simulate a substance that has been found to occur naturally in wort. The above authors reported a decrease in viscosity with increasing maltose concentration (until 5%) and thereafter the viscosity increased with increasing maltose concentration. Using combined static and dynamic light scattering techniques, they also demonstrated that a change (minimum aggregation) in β-glucan structure occurs near 5% maltose. This was attributed to a preferential binding of maltose to β-glucan, which partly breaks up the aggregated β-glucan clusters, as a result of a competitive inhibition mechanism (Grimm et al., 1995; Rees et al., 1982).

The effect of barley β-glucan fiber fractions on wheat starch gelatinization and pasting characteristics have been reported by Symons and Brennan (2004). These authors reported that substitution of 5% wheat starch with the barley β-glucan (63.6-73.5%) fiber fractions decreased the enthalpy of gelatinization and the Rapid Visco Analyzer parameters (peak viscosity, extent of viscosity breakdown, final viscosity) in comparison to the control starch. They postulated that the reduction in pasting characteristics was due to a decrease in starch granular swelling resulting from water being withheld from the starch granules by β-glucan and from a general reduction in starch content of the pastes because of replacement with barley β-glucan.

Our previous studies (Chapter 3) showed that when a low purity (~50%, w/w) barley β-glucan was mixed into an aqueous solution of gelatinized starch (2.5%, w/w), the viscosity did not develop to an extent equivalent to that of a control solution (β-glucan solution prepared without gelatinized starch at identical concentration) (Faraj et al., 2004). Viscosity development of the β-glucan plus gelatinized starch blend solution occurred over a period of time and reached a maximum in approximately 16h. However, the addition of PAA into the solution containing β-glucan plus gelatinized starch blend resulted in a rapid viscosity increase with the time of hydrolysis and the viscosity reached a level that was significantly higher than that of a solution containing only β-glucan (at identical concentration). This indicated that interaction probably occurs between β-glucan and the products of starch α-amylolysis. Furthermore, the above study showed that complete hydrolysis of β-glucan with lichenase destroyed its ability to interact with the hydrolysed starch products. The objective of the current study was to further understand the mechanism of the aforementioned interaction that was responsible for the enhanced viscosity. The study was performed using both low and high purity β-glucan preparations.

4.2 MATERIALS AND METHOD

4.2.1 Materials

Alpha-amylase (EC 3.2.1.1; Porcine pancrease suspension in 2.9 M NaCl containing 3 mM $CaCl_2$), glucose, maltose and maltotriose were purchased from Sigma Chemical Co. (St. Lois, MO). Food grade maltodextrin was obtained from the National Starch and Chemical Company ((Bridgewater, NJ). Fungal protease (EC 3.4.21.62; Deerland fungal protease 400,000) was purchased from Deerland Enzymes (Kennesaw, GA). Lichenase (EC 3.2.1.73; from *Bacillus subtilis*) and β-glucosidase (from *Aspergillus niger*) were purchased from Megazyme International (Wicklow, Ireland). Total starch assay kit that included the enzymes thermostable α-amylase (from *Bacillus licheniformis*) and amyloglucosidase (EC 3.2.1.3; from *Aspergillus niger*) were also purchased from Megazyme International. Termamyl (thermostable α-amylase from *Bacillus licheniformis*) was purchased from Novo Nordisk Biochem, North America Inc. (NC, USA). Sepharose™ CL-6B (in 20% ethanol) was purchased from Amersham Pharmacia Biotech AB (SE-751 84 Uppsala, Sweden). The kit for the determination of total fiber, insoluble and soluble fiber was purchased from Megazyme International (Wicklow, Ireland).

4.2.2 Methods

4.2.2.1 Isolation and purification of starch and beta-glucan from barley flour

Crude (low purity) starch and β-glucan were isolated from pearled hull-less waxy barley flour (CDC-Candle variety) according to the procedure of Vasanthan and Temelli (2002) [Appendix I]. Further purification of crude starch (78%, w/w, purity) and β-glucan (50.7%, w/w, purity) was performed as shown in Figure 4.1.

4.2.2.2 Fractionation of the hydrolysed products resulting from the action of α-amylase on CDC-Candle barley starch

CDC-Candle barley starch (1.0 g) in phosphate buffer (40 ml, pH 9.0) was hydrolysed for different time periods (10 min, 0.5 h, 1h and 5 h) at 37 °C with PPA (3 units/mg starch). The experiment was carried out in a shaker water bath. At the end of the assay period the sample was heated in a boiling water bath for 15 min in order to inactivate the enzyme and then cooled to 30 °C. The hydrolysate was centrifuged at (5,000xg) for 10 min, the residue in the centrifuge tube was recovered and freeze dried. The supernatant was then concentrated in a rotary evaporator to a final volume of about 20 ml. The concentrated sample was chromatographed on a Pharmacia Sepharose™ CL–6B column (2.5x75 cm) at room temperature. Degassed MilliQ water was used as the elution medium. Eluent (3 ml) was collected at 6 min intervals using an auto sample collector and the concentration of total carbohydrates in the fractions was determined using the phenol-sulfuric acid procedure of Dubois et al (1956) [Appendix III]. Contents of the tubes were then pooled according to the peaks identified in the fractions profile Three main peaks were identified and numbered 1, 2 and 3 depending on elution time.

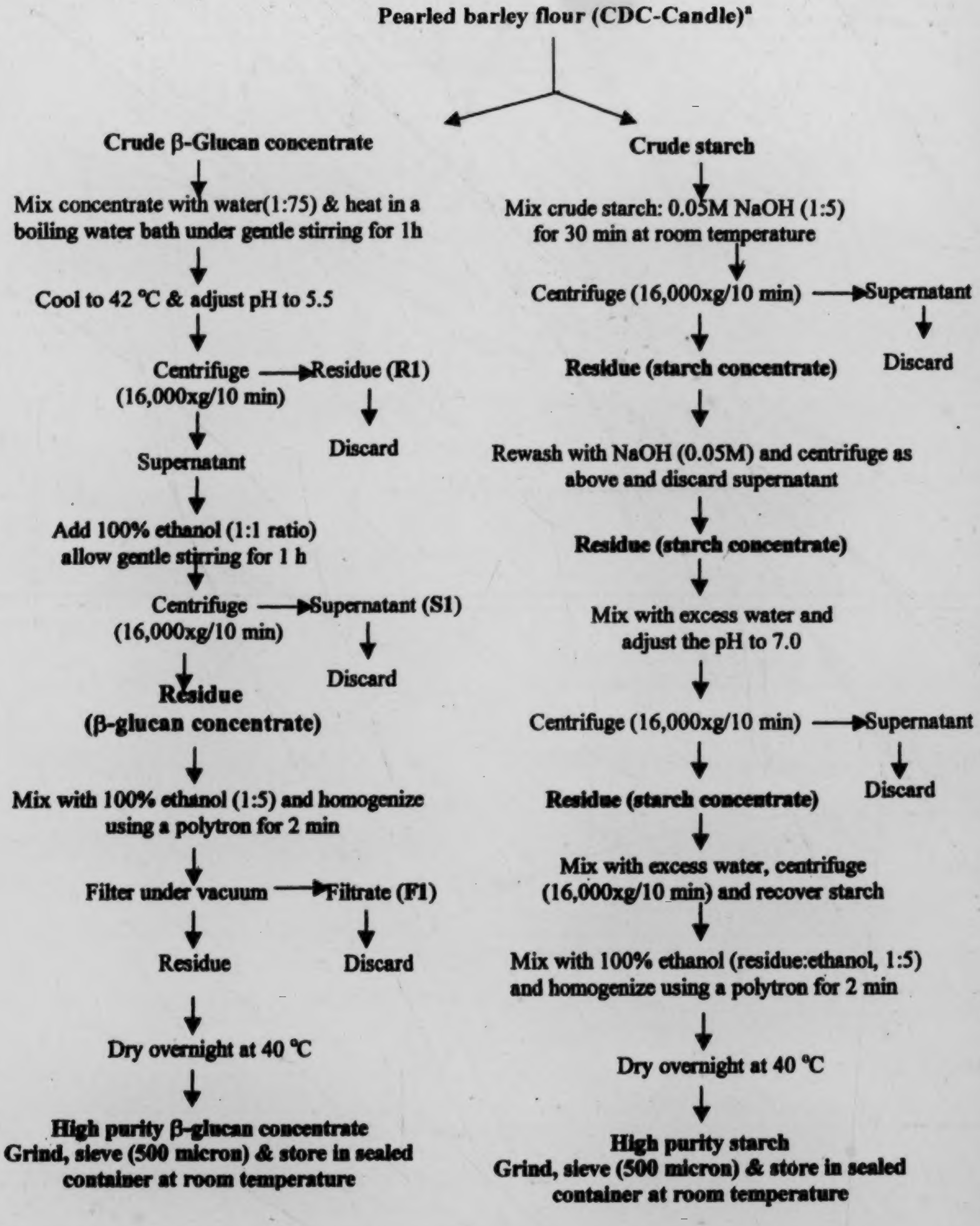
Pearled barley flour (CDC-Candle)[a]
Crude β-Glucan concentrate
Mix concentrate with water(1:75) & heat in a boiling water bath under gentle stirring for 1h
Cool to 42 °C & adjust pH to 5.5
Centrifuge (16,000xg/10 min)
Residue (R1)
Discard
Supernatant
Add 100% ethanol (1:1 ratio) allow gentle stirring for 1 h
Centrifuge (16,000xg/10 min)
Supernatant (S1)
Discard
Residue (β-glucan concentrate)
Mix with 100% ethanol (1:5) and homogenize using a polytron for 2 min
Filter under vacuum
Filtrate (F1)
Residue
Discard
Dry overnight at 40 °C
High purity β-glucan concentrate
Grind, sieve (500 micron) & store in sealed container at room temperature
Crude starch
Mix crude starch: 0.05M NaOH (1:5) for 30 min at room temperature
Centrifuge (16,000xg/10 min)
Supernatant
Discard
Residue (starch concentrate)
Rewash with NaOH (0.05M) and centrifuge as above and discard supernatant
Residue (starch concentrate)
Mix with excess water and adjust the pH to 7.0
Centrifuge (16,000xg/10 min)
Supernatant
Discard
Residue (starch concentrate)
Mix with excess water, centrifuge (16,000xg/10 min) and recover starch
Mix with 100% ethanol (residue:ethanol, 1:5) and homogenize using a polytron for 2 min
Dry overnight at 40 °C
High purity starch
Grind, sieve (500 micron) & store in sealed container at room temperature

Figure 4.1. Isolation and purification of β-glucan and starch. [a] Vasanthan and Temelli, (2002)

Peak 1 represented the fragments with the highest molecular weight, peak 2 contained the medium molecular weight, whereas peak 3 contained the fragments with the lowest molecular weight. The pooled fractions representing each peak were then freeze-dried and stored at room temperature in sealed containers until used. A representative gel permeation chromatogram is shown in Figure 4.2. The chromatograms obtained for the hydrolysates collected at different time interval of hydrolysis were different in appearance (i.e. the proportion of low, medium and high molecular weights and their a time of elution were different).

4.2.2.3 Collection and recovery of by-products during pufication of crude β-glucan

This was performed in order to study the effect of by-product addition on the viscosity of the solution containing purified β-glucan and medium molecular weight (peak 2) fraction of hydrolysed barley starch. Crude β-glucan was once again purified as shown in Figure 4.1. However, this time, the by-products, [residue (R1), supernatant (S1) and filtrate (F1)] were not discarded. The residue (R1) was mixed with milliQ water (250 ml) and homogenized using a Polytron (at medium speed) for 2 min, frozen immediately in liquid nitrogen and then freeze-dried. The supernatant (S1) and filtrate (F1) were combined (as they both would contain water/aqueous ethanol soluble components) and subjected to rotary evaporation, frozen in liquid nitrogen and then freeze dried before storage in sealed containers at room temperature.

4.2.2.4 Sample preparation for viscosity determination

Hydrolysed starch fraction (0.2 g) was weighed in to a screw capped glass tube, mixed with distilled water (8.0 g) and completely dissolved by heating at 80°C for

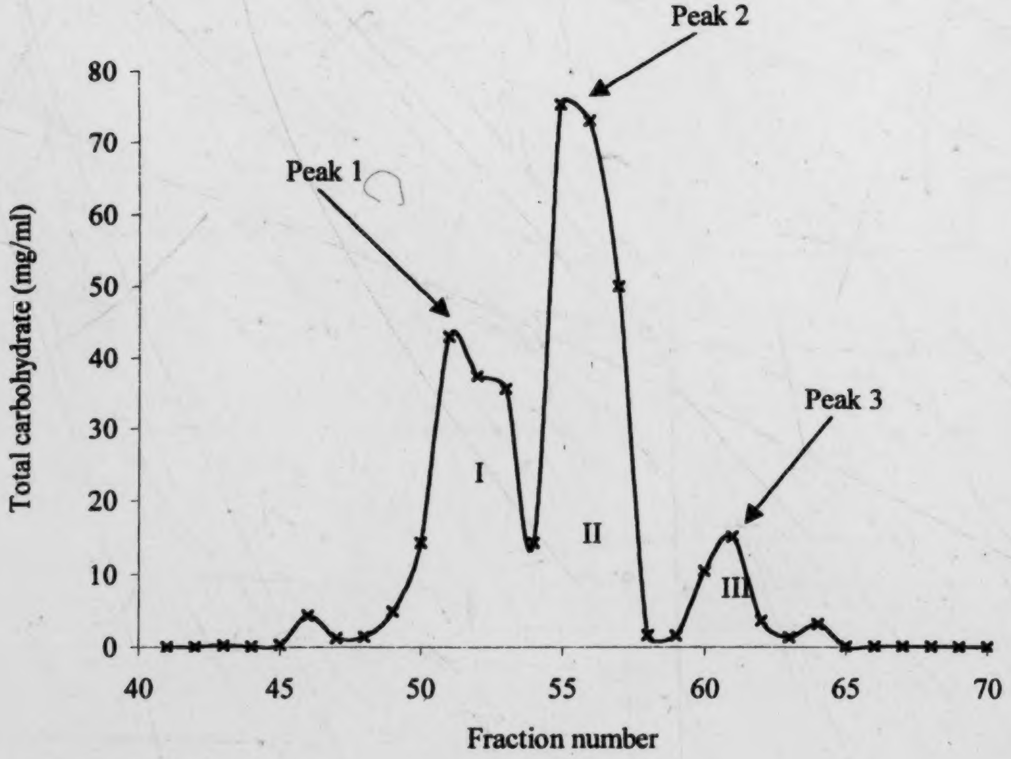

Figure 4.2. Gel permeation chromatography of porcine pancreatic alpha-amylase hydrolysed (5 h) barley (CDC-Candle) starch. Peak 1, 2 and 3 represents the high, medium and lowest molecular weight fractions, respectively.

~10-15 min. β-Glucan (60 mg, 0.75%, w/w) was then added into the solution at 80°C while stirring. When all the β-glucan had dissolved (free of clumps), the solution was cooled to room temperature and left to equilibrate for 1h. Viscosity measurements were then carried out on the solutions (7.0 g) containing the β-glucan and hydrolysed starch fraction. In a parallel experiment, in order to evaluate the effect of heating, solutions containing the β-glucan and hydrolysed starch fractions were heated (80°C) while stirring for 15 min and then cooled to room temperature.

For the study, which investigated the effect of adding back the by-products generated during further purification of crude β-glucan (Fig. 4.1), an amount of each by-product equivalent to the amount that may have been present in the crude β-glucan was accurately weighed and added into the solution containing purified β-glucan (0.75%, w/w) and medium molecular weight fraction of hydrolysed barley starch (2.5%, w/w). The final weight of the solution was 8 g. The solution was then gently stirred for 2h at room temperature before viscosity determination.

4.2.2.5 Determination of viscosity

The viscosity of the solutions prepared under various conditions was determined using a PAAR Physica UDS 200 Universal Dynamic Rheometer (Mebtecknik GmnH Stuttgart, Germany). Six measurements were taken at shear rates of 1.29, 6.45, 12.9, 25.8, 64.5 and 129 s^{-1}, which corresponded to 1, 5, 10, 20, 50 and 100 rpm, respectively. The solutions were weighed (7 g) directly into the rheometer measuring cup. All measurements were made in duplicate and performed mostly at room temperature (20°C) and in some instances at 37°C. The temperature of the samples was controlled using a

Peltier plate, which enabled the temperature of the viscometer to be controlled to within ± 0.1 °C.

4.2.2.6 Effect of glucose, maltose and dextrins on the viscosity of low and high purity β-glucan concentrates

Robyt and French (1970) and Robyt (1984) have shown that the products of PAA hydrolysis are mainly maltose, maltotriose and maltotetraose. Therefore, in order to further understand the influence of glucose, maltose and low molecular weight dextrins on the solution viscosity of low and high purity β-glucan concentrates, authentic samples (glucose, maltose, maltotriose and maltodextrins) were obtained and investigated according to the same procedures and identical concentrations as that used for the PAA hydrolysed starch fractions.

4.2.2.7 Chemical analysis

Moisture, lipid, protein and starch contents were determined according to the standard AACC (2000) procedures. Total, insoluble and soluble fibers were determined according to the Megazyme procedure (AACC Method 32-07, 2000). Starch and β-glucan contents were determined according to the total starch assay (AACC Method 76.13, 2000) and mixed-linkage β-glucan assay (AACC Method 32-23, 2000) procedures, respectively.

4.2.2.8 Statistical analysis

All trials were performed at least in triplicate and chemical analyses were performed in duplicate. Analysis of variance of the data was performed using the General Linear Model (GLM) procedure of SAS Statistical Software Version 8 (SAS

Institute, 2000). Multiple comparisons of the means were performed using the Tukey's test at $P \leq 0.05$ level.

4.3 RESULTS AND DISCUSSIONS

4.3.1 Proximate composition

Most purification protocols for β-glucan (Wood et al., 1978; Dawkins and Nnanna, 1993; Westerlund et al., 1993; Burkus and Temelli, 1998; Lazaridou et al., 2003; Vaikousi et al., 2004) start with grain flour or bran as raw material and employ treatments with thermostable α-amylase and protease followed by dialysis or alcohol precipitation to obtain a high purity β-glucan gum concentrate. In this study, the preparation of high purity β-glucan concentrate was carried out using the low purity β-glucan concentrate (as raw material) obtained as described earlier (Vasanthan and Temelli, 2002). The high purity β-glucan thus obtained had a purity of 87.5% (db) as shown in Table 4.1. Preliminary studies in our lab showed that the use of termamyl (a thermostable α-amylase) in the preparation of low purity β-glucan concentrate (50.7%, db) resulted in a β-glucan concentrate with low residual starch (~5%, w/w). Also, α-amylase activity in the concentrate was not detected as the process involved an enzyme inactivation step (acidified (pH3-3.5) fiber slurry was boiled for 15 min). In this study, in order to ensure minimal starch content in the high purity β-glucan, the low purity β-glucan was treated with amyloglucosidase, which can be totally inactivated by a simple heat treatment. The amyloglucosidase treatment resulted in a gum with less than 1% starch. The soluble (SDF) and insoluble (IDF) dietary fiber contents of the low purity β-glucan concentrate were 51.85 and 31.9%, respectively.

Table 4.1. Proximate composition (% w/w, db) of low and high purity barley β-glucan

Material	β-glucan	Starch	Protein	Lipid	Ash	SDF[1]	IDF[2]
Low purity β-glucan concentrate	50.7±0.8	8.6±0.11	5.6±0.01	0.07±0.0	0.43±0.01	51.9±1.2	31.9±1.3
High purity β-glucan concentrate	87.5±1.1	0.97±0.01	1.8±0.0	0.05±0.0	0.32±0.01	89.3±2.1	8.2±1.1

Values are means of duplicate determination

[1] SDF-Soluble dietary fiber

[2] IDF-Insoluble dietary fiber

The purification procedure used in this study increased the amount of SDF to 89.3% (w/w), most of which was β-glucan (87.5%, w/w).

4.3.2 Gel permeation chromatography of hydrolysed starch fractions and their interaction with low and high purity β-glucan

The starch hydrolysates collected at 10 min, 0.5h and 1h gave a residue upon centrifugation, which was collected separately and freeze dried. However, the hydrolysate collected at 5h, did not have any residue. The effect of the hydrolysed starch fractions on the viscosity of gelatinized starch plus high purity β-glucan solution is presented in Table 4.2. The viscosity measurement at 37°C and at low shear rates mimics the viscosity anticipated in the human intestinal tract, while the measurement at 20°C (generally represents the room temperature in most places) mimics the expected viscosity in a food product.

The viscosity of the solutions was higher at 20°C than at 37°C (Table 4.2). This was expected as temperature inversely affects viscosity of most liquid materials (Dawkins and Nnanna, 1995). The viscosity of the samples containing the hydrolysed starch fractions (low, medium and high molecular weights) and/or residue plus high purity β-glucan was generally lower than that of the control which contained gelatinized non-hydrolysed starch plus high purity (87.5%, w/w) β-glucan. In addition, the effect of heating (80°C for 15 min) of the samples containing high purity (87.5%, w/w) β-glucan and 5h hydrolysate (no insoluble residue) fractions was investigated. The purpose of heating was to observe the influence of heat on β-glucan-starch fractions interaction. Heating of the samples containing fractions from the 5h hydrolysate slightly increased the

Table 4.2. The influence of porcine pancreatic α-amylase hydrolysed (at different time intervals) barley starch fractions on the viscosity of high purity (87.51%, w/w, purity) β-glucan solution (0.75%, w/w).

Time of Hydrolysis	Shear rate (1/s)	Viscosity (mPa.s)									
		Control[1]		BG + HMWSF[2]		BG + MMWSF[2]		BG + LMWSF[2]		BG + Residue[2]	
		20 °C	37 °C	20 °C	37 °C	20 °C	37 °C	20 °C	37 °C	20 °C	37 °C
10 min	1.29	1070^{a}	583^{e}	1035^{b}	560^{f}	972^{c}	519^{g}	883^{d}	527^{g}	1035^{b}	506^{h}
	12.9	676^{b}	420^{e}	659^{b}	407^{f}	630^{c}	383^{g}	591^{d}	411^{ef}	699^{a}	395^{f}
	129	309^{a}	220^{b}	304^{a}	216^{bc}	296^{a}	208^{c}	288^{a}	197^{cd}	292^{a}	190^{d}
0.5h	1.29	1070^{a}	583^{e}	969^{c}	476^{g}	958^{c}	464^{g}	1040^{b}	514^{f}	910^{d}	453^{gh}
	12.9	676^{b}	420^{d}	668^{b}	375^{f}	660^{b}	369^{f}	702^{a}	396^{e}	657^{bc}	365^{f}
	129	309^{a}	220^{c}	262^{b}	182^{d}	262^{b}	181^{d}	270^{b}	188^{d}	263^{b}	182^{d}
1h	1.29	1070^{a}	583^{e}	959^{d}	509^{g}	1040^{b}	552^{f}	IS[4]	IS	999^{c}	485^{h}
	12.9	676^{a}	420^{d}	628^{c}	381^{e}	664^{b}	406de	"	"	679^{a}	383^{e}
	129	309^{a}	220^{c}	298^{ab}	209^{c}	310^{a}	219^{c}	"	"	265^{b}	184^{d}
5h	1.29	1070^{a}	583^{e}	1016^{c}	552^{f}	1031^{b}	539^{g}	875^{d}	452^{h}	NS[5]	NS
	12.9	676^{a}	420^{d}	627^{b}	382^{e}	636^{b}	354^{f}	555^{c}	297^{g}	"	"
	129	309^{a}	220^{c}	303^{a}	217^{c}	254^{b}	175^{d}	240^{b}	160^{e}	"	"
5h (Heated @ 80^{0}C)	1.29	1150^{a}	691^{e}	1045^{c}	580^{f}	1110^{b}	590^{f}	1018^{d}	538^{g}	"	"
	12.9	754^{a}	512^{d}	636^{c}	384^{f}	684^{b}	395^{e}	630^{c}	352^{g}	"	"
	129	281^{a}	221^{c}	256^{b}	176^{e}	266^{b}	218^{d}	257^{b}	176^{e}	"	"

The standard deviations of all determinations were within ±10; Viscosity values followed by different superscripts in each row are significantly different ($p<0.05$) by Tukey's HSD test. [1]Control solution containing β-glucan (0.75%, w/w) and gelatinized-non-hydrolysed starch (2.5%, w/w). [2]Test solutions containing β-glucan (0.75%, w/w) and PAA hydrolysed and gel chromatographed starch fractions (2.5%, w/w) or residue (2.5%, w/w). HMWSF, MMWSF, LMWSF = high, medium and low molecular weight starch fractions, respectively.
[4]NS = No [3]IS = Insufficient sample; [4]NS = No residue..

viscosity in all samples but did not influence the above trend, i.e the viscosity of the samples containing high purity (87.5%, w/w) β-glucan plus 5h hydrolysate isolated fractions was still lower than the control.

The small increase in viscosity due to heating may be attributed to the improved solubilization of and interactions among hydrolysed starch and β-glucan molecules. The low and high molecular weight fractions resulting from the action of α-amylase on barley starch for 5h did not significantly influence the solution viscosity of low purity (50.7%, w/w) β-glucan concentrate (Table 4.3). However, a significant increase in viscosity at low shear rates was observed when the medium molecular weight fraction was added into the low purity (50.7%, w/w) β-glucan concentrate. For instance, at a shear rate of 1.29 s^{-1} the slurry containing the medium molecular weight fraction had a viscosity of 6840 and 9840 mPa s at 37 and 20°C, respectively. The corresponding values for the controls at 37° and 20°C were 5970 and 7520 mPa s, respectively.

The reduction in viscosity upon addition of hydrolysed starch fractions to high-purity (87.5%, w/w) β-glucan (Table 4.2) may be attributed to the influence of sugar and/or dextrin molecules on network formation, which is essential for viscosity development of β-glucan in aqueous systems. Whereas, the increase in viscosity (Table 4.3) in the sample that contained medium molecular weight hydrolysed starch fraction (resulting from the action of α-amylase on barley starch for 5h) and low-purity (50.7%, w/w) β-glucan suggested that there may be a non-β-glucan component in the low purity (50.7%, w/w) β-glucan concentrate that enhances β-glucan network formation.

Table 4.3. The influence of PAA-hydrolysed (5 h) starch fractions on the solution[1] viscosity of crude low purity β-glucan concentrate [2].

Shear rate 1/s	Viscosity (mPa.s)							
	Control[3]		BG + HMWSF[4]		BG + MMWSF[4]		BG + LMWSF[4]	
	20 °C	37 °C	20 °C	37 °C	20 °C	37 °C	20 °C	37 °C
1.29	7520±60^b	5970±42^d	7960±11^b	5770±40^d	9840±41^a	6840±70^c	7920±13^b	6040±16^d
6.45	3700±27^b	2740±18^d	3630±10^b	2680±20^d	4340±40^a	3060±10^c	3600±35^b	2630±34^d
12.9	2340±16^a	1860±11^b	2320±7^a	1830±5^b	2470±30^a	2000±30^b	2300±13^a	1800±17^b
25.6	1410±10^a	1180±8^b	1420±6^a	1170±5^b	1470±1^a	1240±2^b	1410±4^a	1160±6^b
64.5	710±4^a	610±4^b	710±3^a	600±2^b	730±1^a	620±1^b	710±0^a	600±1^b
129	410±3^a	350±2^b	420±2^a	360±2^b	420±0^a	370±1^b	420±0^a	360±0^b

The viscosity values followed by different superscripts in each row are significantly different ($p<0.05$) by Tukey's HSD test.

[1] The β-glucan concentration of the solution is 0.75%, w/w.

[2] The β-glucan content of the concentrate is 50.7%, w/w.

[3] Control solution containing β-glucan (0.75%, w/w) and gelatinized, PAA hydrolysed and gel chromatograph starch fractions (2.5%, w/w).

[4] HMWSF, MMWSF, LMWSF = High, medium and low molecular weight starch fractions (2.5%, w/w), respectively.

4.3.3 Effect of glucose, maltose and dextrins on the viscosity of low and high purity β-glucan concentrates

The effect of glucose, maltose, maltotriose and maltodextrins on the solution viscosity of high purity β-glucan concentrate is presented in Table 4.4. The viscosity of high purity β-glucan concentrate decreased on the addition (2.5%, w/w) of glucose, maltose and the dextrins. However, the viscosity decreased only slightly, when the control [(β-glucan concentrate (0.75%, w/w) plus gelatinized starch (2.5%, w/w)] and the β-glucan concentrate containing the added sugars were heated for a short period of time (80°C/15min) and then cooled to room temperature before viscosity measurements. These results are in close agreement with those of Grimm et al. (1995), who reported that a decrease in viscosity of β-glucan on addition of maltose (2.5-5.0%, w/v), but thereafter the viscosity increase with increasing maltose concentration (up to 10 %, w/v)

Autio et al. (1987) and Dawkins and Nnanna (1995) reported an increase in viscosity of aqueous β-glucan solution with addition of sucrose. The sucrose concentrations (25 and 50%, w/v) used in their study were much higher than that used in this study (2.5%, w/w). However, at higher sucrose concentrations (>65%) they observed a decrease in viscosity of the β-glucan solutions. Similar effects were reported by Elfak et al. (1977) for guar and locust bean gum solutions, who suggested that addition of sugar at high concentration decreases viscosity by restricting the hydration and extension of β-glucan molecules. In this study, the viscosity of low purity (50.7%, w/w) β-glucan concentrate increased slightly on the addition (2.5%, w/w) of glucose, maltose and dextrins (Table 4.5). However, the extent of this increase was not significant (at P<0.05).

Table 4.4. The effect of glucose, maltose and dextrins standards (2.5%, w/w) on the viscosity of high purity β-glucan concentrate (0.75%, w/w: 87.51% purity)[1]

Material	Shear rate (1/s)	Viscosity (mPa.s)									
		BG Control[2]		BG+Glucose		BG+Maltose		BG+Maltotriose		BG+Maltodextrins	
		20 °C	37 °C	20 °C	37 °C	20 °C	37 °C	20 °C	37 °C	20 °C	37 °C
Unheated	1.29	1070^{a}	583^{d}	995^{b}	530^{e}	974^{c}	514^{e}	957^{c}	523^{e}	991^{b}	534^{e}
	12.9	676^{a}	420^{d}	654^{b}	351^{de}	641^{bc}	357^{d}	635^{c}	353^{d}	692^{a}	344^{e}
	129.0	309^{a}	220^{e}	215^{d}	167^{f}	253^{c}	175^{f}	252^{c}	174^{f}	269^{b}	194^{de}
Heated[3]	1.29	1150^{a}	691^{d}	1104^{bc}	641^{f}	1090^{c}	630^{fg}	1080^{bc}	625^{g}	1130^{ab}	660^{e}
	12.9	754^{a}	512^{c}	701^{b}	401^{d}	725^{ab}	411^{d}	716^{ab}	406^{d}	740^{a}	400^{d}
	129.0	281^{a}	221^{b}	275^{a}	189^{b}	275^{a}	192^{b}	272^{a}	190^{b}	295^{a}	197^{b}

[1] The standard deviations of all determinations were within ± 10

[2] Control solution containing β-glucan (0.75%, w/w) and gelatinized starch (2.5%, w/w)

[3] After solubilization the slurry was heated at 80°C for 15 min

Means at specific temperature within a row with different letters are significantly different ($P<0.05$)

Table 4.5. The effect of glucose, maltose and dextrins on the viscosity of low purity β-glucan concentrate (at 0.75%, w/w: 50.7% purity)[1].

Shear rate (1/s)	Viscosity (mPa.s)									
	Control[2]		BG + Glucose[3]		BG + Maltose[3]		BG + Maltotriose[3]		BG + Maltodextrin[3]	
	20 °C	37 °C	20 °C	37 °C	20 °C	37 °C	20 °C	37 °C	20 °C	37 °C
1.29	7940±6	6270±8	8100±9	6360±10	8160±21	6520±10	8050±14	6250±10	8200±10	6660±10
6.45	3880±3	2860±5	3730±3	2840±2	3790±6	3030±5	3670±28	2870±6	3960±20	3020±5
12.9	2450±2	1940±5	2370±2	1900±1	2520±2	2020±5	2310±2	1870±3	2510±4	2030±4
25.8	1480±1	1230±2	1450±0	1200±2	1530±1	1280±2	1390±6	1170±5	1520±1	1280±5
64.6	730±1	620±1	730±0	620±0	770±0	660±0	700±0	600±5	760±0	650±1
129	430±0	370±0	430±0	370±0	450±0	390±0	410±0	350±0	450±0	390±10

[1] Means of duplicate determinations ± standard deviation.

[2] Control solution containing β-glucan (0.75%, w/w) plus gelatinized starch (2.5%, w/w).

[3] Glucose, maltose and dextrins concentrations - 2.5%, w/w.

At a given shear rate and specific temperature the viscosity of the interaction between β-glucan and glucose, maltose and dextrins were not significantly different ($P \geq 0.05$).

4.3.4 The influence of by-products isolated during purification of low purity β-glucan on the solution viscosity of purified β-glucan in the presence of the medium molecular weight fraction of hydrolysed barley starch.

During the purification of crude β-glucan (Fig. 4.1), the product (pure β-glucan and by-products) recovery based on the original weight of raw material (crude β-glucan) was 88% (w/w). The yield of purified β-glucan and by-products [residue (R1), supernatant (S1) and filtrate (F1)] were 54.5% (w/w), 26.2% (w/w) and 7.4% (w/w) (supernatant and filtrate were collected together), respectively. The purified β-glucan concentrate had a β-glucan content of 87.5% (w/w). Proportionate amounts of by-products (i.e., 26.2% for R1 and 7.4% for S1+F1) were added back to the solution containing purified β-glucan and the medium molecular weight fraction of 5h hydrolysed barley starch. The effect of by-product addition on solution viscosity is presented in Table 4.6.

The control and the test solutions containing the by-product fractions displayed pseudoplastic/shear-thinning behavior over the shear rates of 1.29-129 s^{-1}. Adding and mixing of the S1+F1 fraction into the control solution (during its preparation) increased the viscosity (determined at 1.29 s^{-1}) significantly from 1110 mPa s to 1865 mPa s. Viscosity of the control solution was not influenced by the addition of R1 fraction. However, a moderate but significant increase in viscosity (from 1110 mPa s to 1360 mPa s) was observed when the R1 and S1+F1 fractions, were added together into the control solution during its preparation. Similar trends were also observed at a shear rate of 12.9 s^{-1}. Interestingly, at a higher shear rate (129 s^{-1}), none of the by-products influenced the viscosity of the control solution.

Table 4.6. The influence of by-products isolated during purification of β-glucan[1] on the viscosity of purified β-glucan[2] in the presence of the medium molecular weight PAA hydrolysed barley (CDC Candle) starch fraction.

Shear rate	Viscosity (mPa s)			
1/s		By-product fractions[1]		
	Control[3]	Supernatant	Residue	Residue + Supernatant
1.29	1031±10^{c}	1865±20^{a}	1165±20^{c}	1360±23^{b}
12.9	636±5^{e}	795±5^{d}	648±8^{e}	717±7^{d}
129	254±1^{f}	262±1^{f}	260±2^{f}	265±2^{f}

Values are means of duplicate determinations ± standard deviations.

[1] See Fig. 1

[2] 0.75%, w/w.

[3] Control = High purity β-glucan concentrate (0.75%, w/w) plus medium molecular weight fraction of 5h hydrolysed barley starch (2.5%, w/w).

It has been reported by Izydorczyk and MacGregor (2000), that there is evidence of some intermolecular interactions between β-glucan and arabinoxylans. Our results indicate that the third non-β-glucan component that enhances solution viscosity mainly exists in the S1+F1 fraction of the by-products. In addition, the viscosity enhancing property of this component disappeared at shear rates exceeding 12.9 s^{-1}.

Carriere and Inglett (1999) investigated the rheological properties of several β-glucan/amylodextrin blends (Oatrim®). The Oatrim® blend with 10% β-glucan by weight exhibited shear-thickening behavior at 25 °C above a critical shear rate of 30 s^{-1}, however, an Oatrim® with 5% β-glucan did not show any shear-thickening. Furthermore, when the β-glucan content was increased to 24%, two regions of shear thickening was observed in the Oatrim® blend with critical shear rates of 30 and 100 s^{-1}. The authors concluded that the shear thickening regions are due, in part, to a complex interaction between β-glucan and amylodextrin components of Oatrim® at or above a critical level of β-glucan. Our results showed that low purity β-glucan may have interacted with the hydrolytic products of α-amylase to enhance solution viscosity but we did not observed any shear thickening.

4.4 CONCLUSIONS

The influence of PAA hydrolysed barley starch fractions (low, medium and high molecular weight) on the viscosity of low and high purity β-glucan was studied. A small but significant decrease in viscosity was observed when the low molecular weight hydrolysed starch fraction was added to high purity β-glucan. However, the viscosity of the low purity β-glucan increased significantly in the presence of the medium molecular weight hydrolysed barley starch fraction, but remained unchanged in the presence of the low and high molecular weight fractions. This suggested that some non β-glucan components (removed during further purification) in the low purity β-glucan may have been the causative factor responsible for the increase in viscosity observed on the addition of medium molecular weight hydrolysed starch fraction to low purity β-glucan.

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Chapter 5

THE EFFECT OF EXTRUSION COOKING ON β-GLUCAN CONTENT AND RESISTANT STARCH FORMATION IN WAXY AND REGULAR BARLEY FLOURS

5.1 INTRODUCTION

Barley is the fourth major cereal crop produced in the world. Total world barley production is 132 million metric tonnes (FAO, 2002) of which 13.5 million tonnes was produced in Canada. Barley has previously been utilized mainly for malting and brewing and as animal feed. Very little of this is used for human food and value-added processing. Its utilization in Canada is feed ~55-60%, malting and brewing ~25-30%, seed ~2-5% and food and value added ~2-5% (Alberta Barley commission, Calgary, AB. 2002, Personal communication). Studies have shown that barley flour has high content of dietary fiber and high proportion of soluble fiber especially β-glucan. It is therefore becoming an important cereal crop from a nutritional and functional point of view. There is a need to explore the possibility of increasing consumption of barley and barley products for human food and value-added products.

Resistant starch (RS) has been defined by the European Flair Concerted Action on Resistant Starch (EURESTA) as the starch or products of starch degradation that escapes digestion in the small intestine of healthy individuals and may be completely or partially fermented in the colon (Englyst et al., 1992). Resistant starch has been classified into four categories: physically inaccessible to pancreatic alpha-amylase (RS1), amylopectin crystals of uncooked native starch granules (RS2), retrograded starch (amylose crystals) (RS3) (Englyst and Cummings, 1987) and thermally or chemically modified starch (RS4) (Eerlingen and Delcour, 1995). Resistant starch can be found in both raw and processed food materials. Food processing which involve heat and moisture, in most cases destroys RS1 and RS2 but may form RS3. Some RS2 e.g in high amylose maize starch (HAMS) has been reported to be heat stable and can retain its resistance under commonly used food processing conditions (Thompson, 2000; Wang et al., 2001). It has been reported (Björck et al., 1986; Raben et al., 1994; Muir et al., 1995; Brown, 1996) that resistant starch has both physiological and health benefits in humans. RS3 is analyzed as an insoluble dietary fiber but physiologically acts as a soluble dietary fiber. RS3 is a non-caloric ingredient and does not contribute to an increase in blood glucose (Ranhotra et al., 1996a) but decreases post-prandial glucose and insulin response and is therefore ideal for diabetics (Ranhotra et al., 1996b; Haralampu, 2000). Fermentation of RS3 in the colon results in production of high concentration of short chain fatty acids (SCFA) including butyrate (Huth et al., 2000), which has been associated with lowering of cancer risk by deactivating toxic compounds (Wollowski et al., 2001). Butyrate may also inhibit the genotoxic activity of nitrosamides and hydrogen peroxide in human colon cells (Wollowski, et al., 2001). RS3 has been shown to significantly lower blood cholesterol and triglycerides in hamsters (Ranhotra et al., 1996b) but not in human beings (Heijnen et al., 1996)

Several *in vitro* procedures for the quantification of RS have been proposed or used (Englyst et al., 1982; Berry, 1986; Björck et al., 1986; Champ, 1992; Goni et al., 1996; McCleary and Monaghan, 2002). Differences exist in the pH of incubation, enzymes used and the time/temperature combination of the reactions. The amount of RS reported will therefore vary for the different procedures. A collaborative inter-laboratory study (Champ, 1992), comparing RS determining procedures using same samples, reported that the procedure involving incubation of samples with pancreatin at 37°C for 16 h (Berry, 1986) showed higher RS yields for almost all the samples tested than the procedure where the sample had to be heated with the enzyme (thermostable alpha-amylase) at 100°C (Björck et al., 1986).

The resistant starch determination methodology used in the present study involved a heating step with thermostable alpha-amylase at 100°C. Because of this high temperature heating step, the methodology can be regarded as measuring RS3. *In vitro* RS quantification involves determination of RS outside a living organism while *in vivo* methodology involves using ileostomy subjects or the intubation procedure. *In vitro* RS quantification values should be as close as possible to the *in vivo* data which measure the amount of starch that is physiologically significant as a dietary fiber. McCleary (2001) proposed that incubation temperatures for α-amylase be in the range of gelatinization temperature for most normal starches in order to realize RS values more in line with the *in vivo* data. Recently, McCleary and Monaghan (2002) developed an *in vitro* procedure for RS determination that gives values close to the *in vivo* data.

Extrusion cooking is an important and popular food processing technique. Cereals are common ingredients in extruded products and barley flour has been incorporated into some extruded human food (Berglund et al., 1994; Dudgeon-Bollinger et al., 1997). Extrusion cooking has been used for processing breakfast cereals, pasta products, dextrinized flour etc (Camire et al., 1990). Some of the advantages that have been attributed to this technique include low cost, high productivity, versatility and unique product shapes. Depending on the intended final product, various temperatures, moisture, shear and screw speed combinations can be used. Extrusion cooking of starchy grain flours causes gelatinization of starch among other physico-chemical and functionality changes the grain components undergo. A number of investigators (Wu and Sarko, 1978; Berry, 1986; Sievert and Pomeranz, 1989; Eerlingen et al., 1993a; Eerlingen et al., 1994; Vasanthan and Bhatty, 1998) have demonstrated that retrogradation of gelatinized starch induces the formation of resistant starch. Most studies (Sievert and Pomeranz, 1989; Eerlingen et al., 1993a, 1993b; Garcia-Alonso et al., 1998) on resistant starch formation have been done on pure starch systems. Few studies have looked at the effect of extrusion cooking in a mixed system (starch in the presence of other components e.g proteins, β-glucan) such as pearled (removal of the outer layer of the grain and germ by abrasive forces) barley flour. Some of these researchers report the formation of RS during extrusion of cereal (corn and barley) grain flour (Unlu and Faller, 1998; Huth et al. 2000) while others have shown no RS formation in barley and rice during extrusion cooking of cereal grain flour (Östergard et al., 1989; Parchure and Kulkarni, 1997). Vasanthan et al. (2002) observed an increase in RS3 in extruded high amylose barley flour and not in low amylose barley flour. The primary objectives of this study were: (i) to further investigate the effect of extrusion cooking on RS formation in pearled barley flour and (ii) concentrate/isolate RS3 from native and extruded flours.

5.2 MATERIALS AND METHODS

5.2.1 Materials

CDC-Candle (waxy) and Phoenix (regular) hull-less barley grains were obtained from Argicore, Calgary, AB and Nakonechney Family Farms, Millet, AB. The grains were pearled to remove 32% of the outer grain layers using a "Satake" pearler (Model-TM05, Satake, Tokyo, Japan). The pearled grains (68% dry weight) were then pin-milled (Alpine Contraplex wide chamber mill Type A 250, Hosokawa Micron Systems, Summit NJ) at the POS Pilot Plant (Saskatoon, SK) to obtain barley flour passing through 0.5mm sieve. Lichenase (from *Bacillus subtilis*) and β-glucosidase (from *Aspergillus niger*) were purchased from Megazyme International (Wicklow, Ireland). Total starch assay kit that included the enzymes thermostable α-amylase and amyloglucosidase were also purchased from Megazyme International. Fungal protease (from *Aspergillus oryzae*) was purchased from Deerland Enzymes Ltd. (Kennesaw, GA, USA).

5.2.2 Methods

5.2.2.1 Extrusion cooking

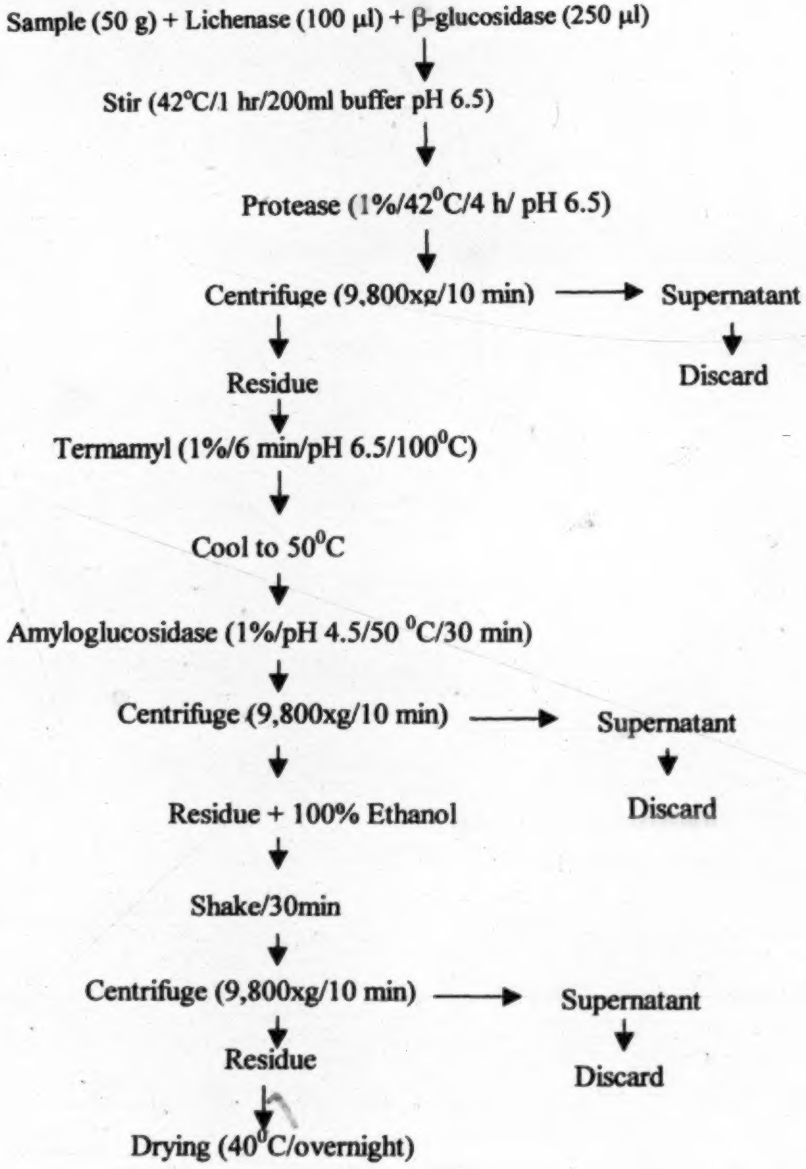
Pearled barley flour from CDC-Candle and Phoenix were extruded in a laboratory-scale conical co-rotating and intermeshing twin-screw extruder (Plasti-corder Digi-system, PL 2200, Brabender Instruments, Inc South Hackensack, NJ). The screws were single flighted and had uniform pitch. The barrel length was 35 cm with a diameter of 31.8/20 mm and the extruder screw had a compression ratio of 3:1. A 4mm die was used. A multi-factorial (5x3x5) design was used for extrusion. The extrusion conditions were temperature (90, 100, 120, 140 and 160°C), moisture content (20, 25, 30 35 and

40%) and screw speed (60, 80 and 100 rpm). The feed section of the barrel was maintained at 60 °C while the temperatures of the compression, metering and die section were changed according to the experimental design. The screw speed was changed after the barrel temperature had stabilized. After extrusion, the samples were cut and cooled to room temperature (about 2h). The extruded samples were then dried overnight in a forced draft oven at 40°C. The dried samples were milled in a Retsch GmbH Ultra Centrifugal mill ZM100 (F. Kurt Retsch GmbH and Co., Hann, Germany) fitted with a 0.5mm screen. The ground samples were kept in airtight containers at room temperature until required for analysis.

5.2.2.2 Resistant starch isolation

Resistant starch (RS3) was isolated according to the procedure outlined in Fig. 5.1. Extruded and native flour samples (50 g) milled and sieved through 0.5 mm were treated with lichenase (100 µl) and β-glucosidase (250 µl) by stirring for one hour at 42°C in a buffer (50 mM, pH 6.5) solution. The ratio of sample to buffer was 1:8. (50 g of flour in 400 ml buffer solution). Fungal protease (1%, w/w) was then added and the whole mixture kept in a shaking water bath at 42°C for 4 h then centrifuged (9,800xg for10 min). The residue was treated with a thermostable α-amylase (Termamyl, 1%, w/w) and incubated in a boiling water bath (100°C) for 6 min. After cooling to 50°C and adjusting the pH to 4.5, amyloglucosidase (1%, w/w) was added and then incubated in a shaking water bath set at 50°C. The mixture was then centrifuged (9,800xg for 10 min). Ethanol (100%) was added to the residue (residue: ethanol 1:4) and shaken for 30 min. Ethanol was then removed by centrifugation (9800xg for 10 min) and the residue dried overnight

Figure 5.1. Resistant starch isolation


Sample (50 g) + Lichenase (100 μl) + β-glucosidase (250 μl)
Stir (42°C/1 hr/200ml buffer pH 6.5)
Protease (1%/42⁰C/4 h/ pH 6.5)
Centrifuge (9,800xg/10 min)
Supernatant
Discard
Residue
Termamyl (1%/6 min/pH 6.5/100⁰C)
Cool to 50⁰C
Amyloglucosidase (1%/pH 4.5/50 ⁰C/30 min)
Centrifuge (9,800xg/10 min)
Supernatant
Discard
Residue + 100% Ethanol
Shake/30min
Centrifuge (9,800xg/10 min)
Supernatant
Discard
Residue
Drying (40⁰C/overnight)

at 40°C. The dried samples were ground in a centrifugal mill to pass through a 0.5 mm sieve and kept in airtight containers at room temperature until they were required for analysis.

5.2.2.3 Resistant starch determination

Resistant starch was determined according to Megazyme International Ltd. (Wicklow, Ireland) procedure that was derived from their total starch assay procedure with minor modifications. Initially the samples (100 mg) were treated with thermostable alpha-amylase [300 units/3 ml of MOPS (contains sodium salt, calcium chloride dihydrate, sodium azide buffer 50 mM, pH 7.0) and boiled for six minutes with vortexing at 2 min interval. The sample was cooled to 50°C and the incubated with amyloglucosidase (AMG, 0.1 ml (20 units)/4 ml sodium acetate buffer, 200 mM, pH 4.5) in a shaking water bath for 30 min. At the end of this period the samples were centrifuged at 9,800xg for 10 min, rather than 3000 rpm (Megazyme procedure). In a preliminary study we observed that increasing the centrifugal speed to 9,800xg resulted in an efficient and quantitative decanting of the supernatant. The residue, which contains RS3 was solubilized by boiling in DMSO (2 ml) for 5 min before being treated once again with thermostable alpha-amylase and AMG. After adjusting the volume to 10 ml with distilled and deionized water, the glucose content was determined by the glucose-peroxidase (GOPOD) assay procedure (as described in the Megazyme total starch assay kit) and used for calculating the amount of resistant starch in the solubilized residue.

5.2.2.4 Chemical analysis

Moisture, ash, lipid and protein contents were determined according to the standard AACC (2000) procedures. The fiber contents (total, soluble and insoluble fiber) were measured using the Megazyme total dietary fiber analysis kit. Amylose content was determined by the method of Chrastil (1987)[Appendix V]. Beta-glucan content was determined using the mixed-linkage beta-glucan assay procedure kit of Megazyme Ltd.

5.2.2.5 Statistical analysis

All experiments were carried out at least in duplicate. Analysis of variance of the results was performed using the General Linear Model (GLM) procedure of SAS statistical software, version 6 (SAS Institute, 1989). Multiple comparison of the means was performed by least significant difference (LSD) test at α=0.05 level.

5.3 RESULTS AND DISCUSSION

5.3.1 Composition of barley grain and flour

The composition of the whole grain and pearled barley flour is shown in Table 5.1 for both Phoenix and CDC-Candle varieties. Starch (63.6 vs 76.6% and 58.5 vs 73.7%, respectively) and β-glucan (3.8 vs 3.9% and 5.9 vs 6.5%, respectively) contents were higher in the pearled barley flour than in the whole grain. This implies that the concentration of these two components across the grain increase from the outside/periphery towards the inside. While for the other components (protein, lipids and ash), the contents were higher in the whole grain than in the pearled flour. This clearly indicates that these components were more concentrated towards the periphery of the

Table 5.1. Proximate analysis (% db)[1] of Phoenix and CDC-Candle grain and flour

Sample	Starch	Protein	β-glucan	Lipid	Ash	Dietary fiber		Amylose content
						SDF[2]	IDF[3]	
Phoenix								
Whole grain	63.63±1.2	13.51±0.5	3.83±0.2	2.13±0.1	1.99±0.1	nd[4]	nd	nd
Pearled barley	76.57±0.4	10.40±0.2	3.87±0.1	1.05±0.0	0.95±0.1	4.00±0.0	2.43±0.6	25.8±0.5
Candle								
Whole grain	58.50±0.7	12.35±0.2	5.94±0.1	2.4±0.12	2.12±0.1	nd	nd	nd
Pearled barley	73.68±0.4	10.21±0.6	6.49±0.2	1.19±0.1	1.18±0.1	6.87±0.1	1.87±0.1	4.81±0.1

[1] All values are the mean of duplicate analyses ± standard deviation

[2] SDF- soluble dietary fiber

[3] IDF- insoluble dietary fiber

[4] nd – not determined

grain and were therefore removed during the pearling process. Similar findings have been reported previously (Yeung et al., 2001; Zheng et al., 2000). Total dietary fiber in CDC-Candle (8.67%) was higher than that in Phoenix samples (6.43%). This can be attributed to the higher soluble dietary fiber (SDF) of CDC Candle (Table 5.1). The insoluble dietary fiber (IDF) was higher in Phoenix (2.43%) than in CDC-Candle (1.87%). The amylose content of Phoenix and CDC-Candle were 25.8% and 4.8%, respectively.

5.3.2 Effect of extrusion cooking on RS3

The effect of extrusion cooking on the formation of RS3 in pearled barley flour from Phoenix and CDC-Candle are shown in Tables 5.2 and 5.3, respectively. Unexpectedly, in both varieties, the total amount of RS3 in the native flour was generally found to be higher than the extruded flour samples. Extrusion at 100°C, increased the RS3 content of Phoenix flour from 46mg/100g to 50-57mg/100g at screw speed and moisture content combinations of 60/30, 60/35, 60/40, 80/30 and 80/35. Whereas, at the other combinations (Table 5.2), the RS3 content was lower (32-36mg/100g) than that of native Phoenix flour. This seems to suggest, that starch fragmentation readily occurs at 100°C at the specified moisture contents (Table 5.2) leading to the formation of amylose chains (with reduced degree of polymerization) that could be incorporated into the crystalline structure of RS3. CDC-Candle behaved slightly differently in exhibiting decreased RS3 contents (17-26 mg/100g) at all temperatures (90-160°C) and screw speed /moisture contents (Table 5.3). This could be attributed to the low amylose contents (Vasanthan et al., 2002).

Table 5.2. The effect of extrusion conditions on RS3 content (mg/100g)[1] of Phoenix flour[2].

Extrusion conditions					
Screw speed (rpm)/ Moisture content (%)	Temperature (^{0}C)				
	90	100	120	140	160
	Amount of RS3 (mg/100g)[1]				
60/20	24±1^{s-w}	32±0^{o-s}	24±2^{s-w}	33±8^{l-r}	17±.6^{w}
60/25	26±1^{p-v}	40±0^{f-m}	31±3^{o-t}	28±3^{p-v}	22±1^{s-w}
60/30	34±0^{l-q}	53±1^{a-d}	33±1^{l-r}	40±4^{f-m}	25±5^{r-w}
60/35	37±1^{h-q}	57±1^{a}	40±1^{f-m}	29±0^{o-v}	21±3^{v-w}
60/40	41±0^{f-l}	54±0^{a-c}	28±2^{p-v}	36±1^{i-o}	28±2^{p-v}
80/20	29±2^{o-v}	36±0^{i-p}	26±2^{q-v}	23±0^{t-w}	22±5^{u-w}
80/25	36±2^{i-p}	50±0^{a-e}	24±4^{s-w}	23±0^{t-w}	24±1^{s-w}
80/30	37±4^{h-o}	50±1^{a-e}	29±1^{o-v}	34±7^{l-q}	22±3^{u-w}
80/35	44±3^{e-l}	56±1^{ab}	26±2^{q-v}	26±2^{q-v}	28±4^{p-v}
80/40	37±4^{h-o}	35±0^{j-p}	30±8^{0-u}	39±8^{f-m}	26±3^{q-v}
100/20	31±1^{o-t}	46±1^{d-h}	21±0^{v-w}	23±1^{t-w}	-nd[3]
100/25	41±1^{f-l}	36±0^{i-p}	31±0^{o-t}	37±4^{h-o}	-
100/30	42±2^{e-k}	33±3^{l-r}	24±1^{s-w}	30±2^{o-u}	-
100/35	32±1^{o-s}	48±1^{b-f}	33±4^{l-r}	23±0^{t-w}	-
100/40	46±1^{c-g}	44±4^{e-i}	29±2^{o-v}	24±4^{s-w}	-

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[1] All values are means of duplicate analyses ± standard deviation.

[2] The RS3 in native Phoenix flour was 46±2 mg/100g.

[3] nd - not determined.

$^{a-w}$Means within the column with different letters are significantly different ($P<0.05$).

Table 5.3. The effect of extrusion conditions on RS3 content (mg/100g)[1] of CDC-Candle flour[2]

Screw speed (rpm)/ Moisture content (%)	Extrusion conditions				
	Temperature (^{0}C)				
	90	100	120	140	160
	Amount of RS3 (mg/100g)				
60/20	$26\pm1^{\text{i-o}4}$	$30\pm3^{\text{f-k}}$	$22\pm0^{\text{m-q}}$	$25\pm1^{\text{j-p}}$	$32\pm1^{\text{d-i}}$
60/25	$19\pm2^{\text{pq}}$	$30\pm1^{\text{f-k}}$	$41\pm1^{\text{a-c}}$	$29\pm1^{\text{f-l}}$	$34\pm0^{\text{d-g}}$
60/30	$17\pm2^{\text{q}}$	$45\pm6^{\text{a}}$	$34\pm0^{\text{d-g}}$	$42\pm3^{\text{ab}}$	$31\pm2^{\text{e-i}}$
60/35	$20\pm2^{\text{o-q}}$	$31\pm2^{\text{e-i}}$	$30\pm0^{\text{f-k}}$	$32\pm3^{\text{d-i}}$	$32\pm1^{\text{d-i}}$
60/40	$26\pm2^{\text{i-o}}$	$31\pm2^{\text{e-i}}$	$23\pm0^{\text{l-q}}$	$30\pm1^{\text{f-k}}$	$31\pm2^{\text{e-i}}$
80/20	$37\pm2^{\text{b-e}}$	$42\pm2^{\text{ab}}$	$38\pm3^{\text{b-d}}$	$26\pm2^{\text{i-o}}$	$33\pm2^{\text{d-g}}$
80/25	$25\pm0^{\text{j-p}}$	$28\pm3^{\text{g-m}}$	$28\pm1^{\text{g-m}}$	$26\pm4^{\text{i-o}}$	$22\pm2^{\text{m-q}}$
80/30	$35\pm3^{\text{c-f}}$	$34\pm2^{\text{d-g}}$	$28\pm1^{\text{g-m}}$	$29\pm1^{\text{f-l}}$	$25\pm2^{\text{j-p}}$
80/35	$27\pm1^{\text{g-n}}$	$33\pm6^{\text{d-g}}$	$31\pm1^{\text{e-i}}$	$26\pm3^{\text{i-o}}$	$47\pm6^{\text{a}}$
80/40	$28\pm1^{\text{g-m}}$	$34\pm1^{\text{d-g}}$	$45\pm4^{\text{a}}$	$32\pm4^{\text{d-i}}$	$24\pm1^{\text{k-p}}$
100/20	$26\pm0^{\text{i-o}}$	$23\pm2^{\text{l-q}}$	$23\pm2^{\text{l-q}}$	$28\pm4^{\text{g-m}}$	nd[3]
100/25	$31\pm0^{\text{e-i}}$	$33\pm2^{\text{d-h}}$	$33\pm2^{\text{d-g}}$	$21\pm1^{\text{n-q}}$	-
100/30	$29\pm2^{\text{f-l}}$	$25\pm1^{\text{j-p}}$	$25\pm1^{\text{j-p}}$	$24\pm1^{\text{l-p}}$	-
100/35	$23\pm2^{\text{l-q}}$	$31\pm4^{\text{e-j}}$	$31\pm4^{\text{e-i}}$	$20\pm1^{\text{o-q}}$	-
100/40	$33\pm0^{\text{d-g}}$	$25\pm1^{\text{j-p}}$	$23\pm2^{\text{l-q}}$	$22\pm1^{\text{m-q}}$	-

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[1] All values are means of duplicate analyses ± standard deviation.

[2] The RS3 in native CDC-Candle flour was 43±1 mg/100g.

[3] nd – not determined.

[4] Means within the column with different letters are significantly different ($P<0.05$).

The decrease of RS3 content at 90°C (Tables 5.2 and 5.3) suggests, that at this temperature, although crystallites forming the structure of RS3 in the native flour are torn apart, no new compensating or additional crystallites are formed between amylose chains (since the extrusion temperature is probably below the threshold for starch fragmentation). The higher decrease in RS3 content at temperatures exceeding 120°C (Table 5.2) suggests melting of the crystallites that form the structure of RS3. Extrusion cooking at the given conditions of temperatures, screw speed and moisture content showed significant differences ($P<0.05$) in the amount of RS3 formed in both barley varieties. However, there was no particular trend observed in the amount of RS3 formed. Amylose content has been shown to have a significant effect on the amount of RS3 formed (Sievert and Pomeranz, 1989, 1990, Eerlingen et al., 1993b). Vasanthan et al. (2002) reported the formation of RS3 in extruded high amylose barley flour but did not observe any RS3 in extruded low amylose barley flour. Their study was conducted at a limited combination of extrusion conditions and in a commercial extruder. The present study investigated a wider range of extrusion conditions. Leloup et al. (1992) demonstrated that amylose contributes more resistant starch formation than amylopectin. These researchers proposed that retrograded amylose gel exhibits a macroporous structure containing filaments of 20±10 nm wide and that these filaments result from the association of segments of amylose chains of specific degree of polymerization (DP) ($26<DP<73$) which are partially organized in a B-type crystalline structure.

In the present study, the RS3 contents of native and extruded Phoenix and CDC-Candle flours were low despite their differences in amylose content. The extrusion conditions, especially the shearing action of the extruder screw, may have caused degradation of the amylose into molecules of smaller DP (<26) that could not be incorporated into a crystalline structure, and therefore resulted in low formation of RS3 (Gomez and Aguilera, 1984; Gidley et al., 1995; Govindasamy et al., 1996). Huth et al. (2000) showed that a higher shearing occurs at low feed moisture content (15%) while Unlu and Faller (1998) reported a negative relationship between formation of RS3 and screw speed. In addition, flour is a complex system, and other components such as proteins, β-glucans, pentosans, etc may interfere with the formation of RS3.

The literature on RS3 formation in extruded cereal flours shows contrasting information. Some researchers have reported formation of RS3 during extrusion of cereal flour, a mixed system. Huth et al. (2000) observed up to 6% RS3 formed during extrusion of barley flour followed by freeze storage at -18°C for 3-7 days. While Unlu and Faller (1998) reported formation of RS3 during extrusion of corn meal blended with high amylose maize starch (14.38% RS for 7.5% citric acid and 30% HAMS) or in the presence of citric acid (5.23% RS3 at 7.5% citric acid). However, some other researchers have reported lack of RS3 formation during extrusion cooking. Östergard et al. (1989) did not observed any RS3 formation during extrusion of barley flour.

Parchure and Kulkarni (1997) reported lower RS3 content after extrusion cooking of rice (3.6%) and amaranth starch (3.4%) compared to pressure cooking (5.4 and 3.6%, respectively). Siljeström et al., (1986) observed a decrease in RS3 in extruded wheat flour and reported that there were no changes in dietary fiber when extruding wheat flour. The amount of RS3 in most commercial extruded grain-based foods is very low (0-0.6%, w/w) (Geltroth and Ranhotra, 2000). Our results further confirm that even under extreme extrusion conditions of high moisture content (20-40%) and very low screw speed (60 rpm) there was very low amounts of RS3 formed, though significant ($p<0.05$). It is only by incorporating other ingredients or optimizing post extrusion conditions e.g freeze storage, that RS3 increase may be observed.

5.3.3 Effect of cooling (4°C) of extruded flour samples on the formation of RS3

Starch gel is a partially crystalline polymer system and retrogradation can be considered as crystallization in an amorphous system (Eerlingen et al. 1993a). Björck et al (1987) observed that autoclaved wheat starch samples that were cooled and refrigerated (4°C) overnight had a higher RS (21.6%) than those that were immediately frozen and lyophilized (16.5%). Extended storage, 7-14 days, of the autoclave cereal samples at 4°C did not change the amount of RS (Berry, 1986; Sievert and Pomeranz, 1989). Resistant starch, as earlier discussed is composed mainly of the B-type crystalline structure. Slade and Levine (1993) showed that the B-type of starch gels containing more than 27% water have glass transition temperatures (T_g) near –5°C. Eerlingen et al. (1993a) observed that at 0°C the nucleation rate of crystalline starch formation was very high and the initial yield of RS increases rapidly. Nucleation takes place above but close to the T_g of a polymer. A number of researchers have shown that retrogradation of gelatinized starch takes place faster under refrigeration (4°C) conditions (Jacobson et al., 1997; Eerlingen et al. 1993a, 1993b; Siervert and Pomeranz, 1989; Berry, 1986). This may be attributed to enhanced nucleation and chain length elongation phase of the double helical structure formation. Therefore, a set of studies were conducted, where the extruded samples were cooled to room temperature and then refrigerated at 4°C overnight before being dried in the oven at 40°C for 24 h. The amount of RS3 formed at 4°C and room temperature is shown in Table 5.4 for CDC-Candle and Phoenix varieties. There was significant difference ($P<0.05$) in the amount of RS3 formed at different extruding conditions. Slightly more RS3 was observed when the samples were kept at 4°C overnight. However, there was no significant difference ($P<0.05$) in the amount of RS3 formed at 4°C compared to those only cooled to room temperature before oven drying. This may also be attributed to the excessive fragmentation of the starch molecules especially the amylose fraction. Due to the shearing effect, the amylose molecules may have been fragmented into sizes that may have been too small for forming the double helical structures of RS3 crystallites.

5.3.4 Isolation of RS3

Hölm et al. (1986) have shown that a large fraction of the starch in raw and boiled wheat is embedded with a protein matrix, thus restricting the availability of starch to α-amylase *in vitro* as illustrated in Fig 5.2. These components (protein and β-glucan) may affect the *in vitro* RS3 determination and should be removed before quantification of RS3. The methodology for isolation/concentration of RS3 is outlined in Fig 5.1.

The amounts of RS3 isolated from the extruded samples are presented in Table 5.5. Higher RS3 contents were observed in the extruded samples before isolation than in the extruded after isolation samples for both varieties. Under the same extrusion conditions more RS3 was isolated from the Phoenix than from CDC-Candle. This could be attributed to the higher amylose content of Phoenix (Table 5.5).

Table 5.4. The RS3 content (mg/100g)[1] of CDC-Candle and Phoenix extruded flours stored at 4 and 25°C.

Extrusion Conditions[2] (°C/rpm/%)	Candle		Phoenix	
	Storage temp		Storage temp	
	25°C	4°C	25°C	4°C
Native flour	46 ± 2	46 ± 0	43 ± 1	46 ± 6
90/100/20	$26\pm0^{l-n}$	$34\pm1^{f-i}$	$31\pm1^{j-o}$	$36\pm0^{h-l}$
90/100/25	$31\pm0^{i-k}$	39 ± 0^{ef}	$41\pm1^{e-i}$	61 ± 1^{a}
90/100/30	$29\pm2^{j-l}$	40 ± 0^{de}	$42\pm2^{e-h}$	60 ± 4^{ab}
90/100/35	$23\pm2^{n-p}$	56 ± 1^{a}	$32\pm1^{j-n}$	$38\pm2^{f-i}$
90/100/40	$33\pm0^{h-j}$	55 ± 1^{a}	$46\pm1^{d-f}$	$56\pm1^{a-c}$
100/100/20	$23\pm2^{n-p}$	46 ± 2^{cb}	$36\pm0^{h-l}$	$31\pm1^{j-o}$
100/100/25	$33\pm2^{h-j}$	$38\pm0^{e-h}$	$23\pm6^{o-p}$	$36\pm2^{h-l}$
100/100/30	$25\pm1^{l-o}$	54 ± 0^{a}	$48\pm1^{c-e}$	$45\pm2^{d-g}$
100/100/35	$31\pm4^{i-k}$	49 ± 2^{b}	$44\pm4^{d-h}$	$41\pm4^{e-i}$
100/100/40	$25\pm1^{i-o}$	$37\pm0^{e-h}$	$45\pm2^{d-g}$	$37\pm4^{g-k}$
120/100/20	$28\pm4^{i-k}$	$38\pm1^{e-h}$	$21\pm0^{p-q}$	$44\pm0^{d-h}$
120/100/25	$21\pm0^{o-p}$	$33\pm0^{h-j}$	$31\pm0^{j-o}$	$42\pm1^{e-h}$
120/100/30	$24\pm1^{m-p}$	41 ± 2^{ed}	$24\pm1^{n-p}$	$37\pm6^{g-k}$
120/100/35	$20\pm0^{p-r}$	$32\pm0^{i-k}$	$33\pm8^{l-m}$	$52\pm1^{b-d}$
120/100/40	$20\pm1^{p-r}$	$37\pm3^{e-h}$	$29\pm3^{k-p}$	$31\pm1^{j-o}$
140/100/20	14 ± 0^{s}	$33\pm1^{g-j}$	$23\pm1^{o-p}$	$41\pm1^{e-i}$
140/100/25	$21\pm1^{o-q}$	45 ± 3^{cb}	$37\pm4^{g-k}$	$26\pm1^{m-p}$
140/100/30	$17\pm1^{q-s}$	41 ± 2^{cd}	$30\pm2^{j-o}$	14 ± 4^{q}
140/100/35	$16\pm1^{r-s}$	39 ± 0^{ed}	$23\pm0^{o-p}$	$28\pm2^{l-p}$
140/100/40	$20\pm1^{n-p}$	48 ± 1^{b}	$24\pm4^{n-p}$	$25\pm1^{m-p}$

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[1] All values are means of duplicate analyses ± standard deviation.

[2] Extrusion conditions: temperature/screw speed/moisture content (^{0}C/rpm/%).

[a-s] For a variety, means within a column with different letters are significantly different ($P<0.05$).

Table 5.5. The RS3 content (mg/100g) [1] in extruded Phoenix and CDC-Candle flours before and after enzymatic isolation

Extrusion Conditions[2] (°C/rpm/%)	Extruded Phoenix flour		Extruded Candle flour	
	Before isolation	After isolation	Before isolation	After isolation
Native flour	46±2	32±1	43±1	11±1
90/100/20	$31\pm0^{d-f}$	19 ± 1^{jk}	35 ± 0^{de}	$8\pm.5^{jk}$
90/100/25	41 ± 1^{b}	18 ± 1^{k}	39 ± 0^{b}	13 ± 1^{hi}
90/100/40	46 ± 1^{a}	$28\pm2^{f-h}$	40 ± 2^{ab}	$6\pm.5^{k}$
100/100/20	36 ± 1^{c}	22 ± 2^{ij}	26 ± 2^{ef}	$6\pm.5^{k}$
100/100/25	46 ± 2^{a}	34 ± 3^{de}	34 ± 2^{d}	15 ± 1^{h}
100/100/40	37 ± 2^{c}	18 ± 1^{k}	38 ± 2^{bc}	$6\pm.5^{k}$
120/100/20	26 ± 1^{gh}	21 ± 1^{jk}	23 ± 2^{fg}	8 ± 1^{jk}
120/100/25	$31\pm1^{d-f}$	19 ± 2^{jk}	32 ± 0^{d}	11 ± 1^{ij}
120/100/40	27 ± 1^{gh}	$29\pm1^{e-g}$	25 ± 1^{ef}	8 ± 1^{jk}
140/100/20	$28\pm0^{f-h}$	20 ± 1^{jk}	28 ± 0^{e}	$6\pm.5^{k}$
140/100/25	37 ± 1^{c}	21 ± 1^{jk}	21 ± 1^{g}	6 ± 0^{k}
140/100/40	27 ± 1^{gh}	25 ± 1^{hi}	20 ± 1^{g}	11 ± 1^{ij}

[1] All values are means of duplicate analyses ± standard deviation.
[2] Extrusion condition represents: temperature/screw speed/moisture content.
[a-k] For each variety, means within the columns with different letters are significantly different ($P<0.05$).

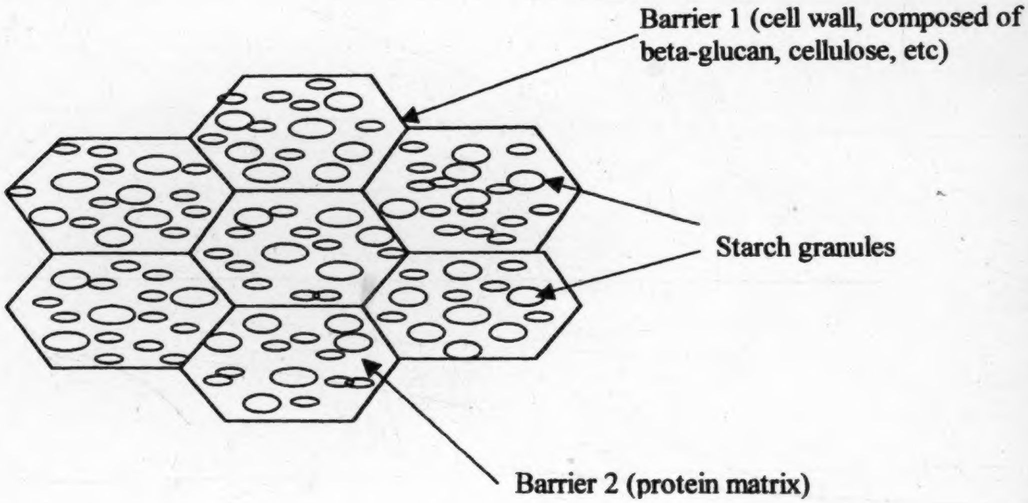
Barrier 1 (cell wall, composed of beta-glucan, cellulose, etc)
Starch granules
Barrier 2 (protein matrix)

Figure 5.2. Cells of barley grains where starch granules are embedded in protein matrix.

The amount of RS3 isolated from the extruded flour samples was unexpected. It was anticipated that by enzymatic hydrolysis of the proteins, β-glucans and non-resistant starches we may concentrate RS3 and therefore increase its quantity in the residue of the isolates.

The objective of the sequential enzymatic treatment was to hydrolyze these components (proteins, β-glucans, and non-resistant starch) in order to concentrate RS3. The RS3 contents of the resulting residue from these sequential enzymatic treatments ranged between 18-34mg/100g and 6-15mg/100g compared to 26-46mg/100g and 20-40 mg/100g for Phoenix and CDC-Candle, respectively, in the extruded (before sequential enzymatic treatment and isolation) samples, as shown in Table 5.5. This implies that RS3 did not concentrate in all the samples analyzed.

The recovery of RS3 from the extruded samples was low and calculated to be about 50%. The data suggested that the either RS3 may become susceptible to the thermostable α-amylase hydrolysis after the other components (β-glucan, protein etc) entrapping it had been hydrolyzed, or the resistant starch content determined by the modified Megazyme method included other types of resistant starches such as RS1 and RS2. However, in a collaborative inter-laboratory study (Champ, 1992) it was shown that the same samples incubated at 37°C for 16 h with porcine pancreatic α-amylase (Berry, 1986) exhibited a higher RS3 content than samples that were treated with thermostable α-amylase at 100°C for 15 min (Björck et al. 1986). As discussed earlier RS3 formation is mainly due to retrogradation of amylose. Retrograded crystallites are very stable and show a melting endotherm at about 150°C (Leloup et al., 1992). The 15 min heat treatment (Björck et al., 1986) or the six minutes (Megazyme procedure used in this study) may be severe enough to cause enzymatic hydrolysis of the retrograded amylose. The low RS3 recovery calculated in this study could be due to cumulative carry-over losses during the isolation process.

5.3.5 Effect of extrusion on β-glucan content

The effect of extrusion conditions on β-glucan content is presented in Table 5.6. There were significant differences ($P<0.05$) in the amount of β-glucan in the extruded products at the various extrusion conditions except at 140°C. However, the differences in the amount after extrusion were small compared to the β-glucan content in native CDC-Candle flour (7.64%, db). The extruded samples did not show any significant differences at 140°C under the various screw speed and moisture content. There was no plausible explanation for this odd occurance.

It has been reported by Jiang and Vasanthan (2000) that extrusion of CDC-Candle flour at 90-140°C and 20-50% moisture content resulted in a higher percentage of β1→ content and lower β1→3 and β1→4 than in the native samples and the trend was more pronounced at >120°C. While the solubility of the extruded samples was higher than the native samples. The above authors postulated that the increase in relative percentage of β1→ and simultaneous decrease in the relative percentage of β1→3 and β1→4 suggested that CDC-Candle β-glucan had fragmented during extrusion cooking. Asp and Björck (1989) have also reported that extrusion cooking can cause a shift from insoluble to soluble fiber.

Table 5.6. The effect of extrusion conditions on β-glucan content (%, db)[1] of Candle flour[2]

	Extrusion conditions				
Screw speed (rpm)/ Moisture content (%)	Temperature (oC)				
	90	100	120	140	160
			Amount of β-glucan (%, db)		
60/20	$7.35\pm.37^{a-c}$	$7.10\pm.24^{a-c}$	$6.92\pm.01^{a-c}$	$7.43\pm.30^{a}$	$7.93\pm.17^{a}$
60/25	$6.95\pm.08^{e}$	$7.34\pm.25^{a-c}$	$6.91\pm.06^{a-c}$	$7.54\pm.27^{a}$	$7.59\pm.13^{ab}$
60/30	$7.02\pm.01^{d-e}$	$7.31\pm.07^{a-d}$	$7.18\pm.17^{a-c}$	$6.88\pm.15^{a}$	$7.23\pm.56^{b-d}$
60/35	$7.23\pm.03^{b-e}$	$7.16\pm.06^{a-e}$	$7.52\pm.35^{ab}$	$7.42\pm.23^{a}$	$7.38\pm.15^{a-c}$
60/40	$7.28\pm.09^{b-e}$	$7.32\pm.46^{a-d}$	$7.29\pm.71^{a-c}$	$7.31\pm.39^{a}$	$7.21\pm.06^{b-d}$
80/20	$7.64\pm.07^{a-c}$	$7.74\pm.08^{a}$	$7.42\pm.06^{ab}$	$7.17\pm.62^{a}$	$7.13\pm.33^{bc}$
80/25	$7.78\pm.13^{ab}$	$6.80\pm.04^{c-e}$	$7.10\pm.04^{a-c}$	$7.41\pm.20^{a}$	$6.46\pm.08^{e}$
80/30	$7.08\pm.04^{de}$	$7.30\pm.03^{a-e}$	$6.88\pm.13^{bc}$	$7.26\pm.21^{a}$	$6.96\pm.06^{c-e}$
80/35	$7.87\pm.19^{a}$	$7.53\pm.40^{ab}$	$7.26\pm.15^{a-c}$	$7.41\pm.25^{a}$	$6.90\pm.08^{a-e}$
80/40	$7.88\pm.11^{a}$	$7.53\pm.35^{ab}$	$6.92\pm.13^{a-c}$	$7.37\pm.33^{a}$	$6.65\pm.15^{de}$
100/20	$7.33\pm.65^{a-c}$	$6.68\pm.05^{de}$	$6.87\pm.10^{bc}$	$7.12\pm.40^{a}$	nd[3]
100/25	$7.16\pm.07^{c-e}$	$7.00\pm.51^{b-e}$	$7.35\pm.78^{a-c}$	$6.93\pm.24^{a}$	“
100/30	$7.39\pm.69^{a-c}$	$6.65\pm.23^{e}$	$6.93\pm.14^{a-c}$	$7.72\pm.11^{a}$	“
100/35	$6.91\pm.07^{e}$	$7.18\pm.10^{a-e}$	$6.62\pm.11^{c}$	$7.18\pm.17^{a}$	“
100/40	$7.56\pm.08^{a-d}$	$7.37\pm.02^{a-c}$	$7.17\pm.02^{a-c}$	$6.91\pm.14^{a}$	“

[1] All values are means of duplicate analyses ± standard deviation.

[2] The β-glucan content in native CDC-Candle flour was 7.64±0.34% (db).

[3] nd – Not determined.

[a-f] Means within the column with different letters are significantly different ($P<0.05$).

5.4 CONCLUSIONS

Extrusion cooking of waxy and regular pearled barley flour under the conditions investigated did not lead to the formation of RS3. Refrigeration at 4°C for 24 h before oven drying of extruded flour samples slightly increased RS3 content. Sequential enzymatic treatment in order to concentrate/isolate RS3 was not successful. However, sequential enzymatic hydrolysis due to the high β-glucan and protein contents in pearled barley flour is required for accurate RS3 quantification. There was a small but significant difference in the amount of β-glucan after extrusion compared to the native unextruded barley flour.

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Chapter 6

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 SUMMARY AND CONCLUSIONS

The influence of low purity (~50%) β-glucan concentrate (BBF) content on the *in vitro* hydrolysis of gelatinized starch by porcine pancreatic α-amylase (PAA) was investigated in this study. In general, starch hydrolysis by PAA decreased with increase in BBF concentration from 0 to 0.75% (w/v), with the exception of increased starch hydrolysis at BBF concentration of 1.0% (w/v). Data showed that the viscosity of the gelatinized starch slurry containing BBF increased with increasing BBF concentration. It was also observed that as the PAA activity progressed, the viscosity of the slurry increased significantly. The decrease in gelatinized starch hydrolysis may be attributed to the increase in viscosity. The increase in viscosity could decrease the rate of diffusion of PAA to gelatinized starch and therefore impede adsorption of enzyme and its catalytic action on starch.

Low purity β-glucan alone had a much higher viscosity than β-glucan/starch mixture. However, the viscosity of gelatinized starch/β-glucan blend increased during the first 16 h and then stabilized thereafter. The delay in viscosity changes can be utilized in the formulation of a product that can be consumed before the full development of viscosity. For instance, a beverage that has both starch and β-glucan, and is still drinkable can be formulated. When starch is hydrolysed in the stomach, the amylolytic products interact with β-glucan increasing the viscosity of the digest thereby enhancing the health benefits of the product. The addition of α-amylase to the gelatinized starch/β-glucan slurry resulted in a rapid increase in viscosity. The increased viscosity was attributed to the interactions among β-glucan and the intermediate fractions of hydrolysed starch.

A simplified BBF purification procedure was employed to obtain a product containing β-glucan, protein, and starch at 87.7%, 1.8% and < 1%, respectively. The high (~88%) purity β-glucan concentrate (BBG) was used to investigate the influence of β-glucan on starch hydrolysis by PAA. The viscosity of BBG (0.75%, w/v) was lower than the viscosity of BBF at the same concentration. There was no change in the viscosity of the BBG/gelatinized starch slurry as PAA action progressed. This was contrary to what had been observed for BBF, where the viscosity of the slurry increased as the PAA action on the gelatinized starch hydrolysis progressed. I feel that some of the components in the supernatant or residue discarded during the purification of BBF, may have been responsible for the increased viscosity during the PAA-assisted starch amylolysis in the presence of β-glucan.

Fractionation of PAA hydrolysed gelatinized starch using a column packed with Sepharose CL-6B yielded three major (high, medium and low molecular weight) fractions. Incorporation of these fractions into the gelatinized starch/BBG slurry did not significantly change the viscosity of the slurry during the PAA-assisted amylolysis of starch. However, when the fractions were incorporated into the gelatinized starch/BBF slurry, the medium molecular weight fraction (MMWF) caused a significant increase in viscosity at both 20 and 37°C. The increase in viscosity in the sample containing MMWF may be attributed to complex interactions among β-glucan and the hydrolytic components of the fraction.

The influence of glucose, maltose, maltotriose and maltodextrins on the viscosity of PAA-hydrolysed gelatinized starch/β-glucan slurry was also investigated. The sugar/dextrins used in this study did not affect the viscosity of the BBF and BBG slurries. The concentration of sugars/dextrins (2.5%, w/w) used in this study may have been too low to cause any complex interactions with β-glucan.

An attempt was made to screen all the fractions obtained during the purification of β-glucan for viscosity-enhancing properties in MMWF/BBG slurry. In addition to BBG, two other major fractions [supernatant (aqueous ethanol soluble) and residue] were collected with a recovery of 88%. The residue did not significantly ($P<0.05$) influence the viscosity of the MMWF/BBG slurry. However, the supernatant and the supernatant plus residue fractions significantly increased the viscosity of MMWF/BBG slurry. It is evident, that in addition to starch hydrolytic products, there are other components that act synergistically to enhance the viscosity of the slurry. These components may have been present in the supernatant and were either lost during the purification process or were converted into forms with no synergistic properties.

In a mixture of lichenase hydrolysed-β-glucan and PAA hydrolysed-gelatinized starch slurry, the degree of α-amylolysis was extensive and hence the viscosity of the slurry was significantly reduced. This suggests that the effectiveness of β-glucan in reducing amylolysis of gelatinized starch is greatly dependent on the influence of β-glucan chain length on the viscosity of the resulting slurry.

The objective of fractionation/isolation of raw food materials is to obtain fractions/isolates that are of a high value and purity. The purity of BBG (87.5%) prepared in this study was high, however, unexpectedly it did not cause any significant increase in the viscosity of PAA hydrolysed starch/β-glucan slurry. Purified BBG solution (0.75%, w/w) had a lower viscosity compared with BBF at the same concentration. However, both the crude BBF and the purified BBG may find application in different food and non-food applications. For instance, BBF can be used in the formulation of food products where the changes in viscosity can be an advantage both as a functional (thickening) and nutritional (decreased amylolysis) attribute, whereas the BBG can be utilized in the pharmaceutical industry where medium viscosity and high purity is essential.

Extrusion cooking was used in this study as an attempt to decrease native starch content and to increase resistant starch in the extruded barley flour product. Extrusion conditions used in this study did not increase the RS3 content of the extruded product. There were no indications that new RS3 was formed but its contents generally decreased. However, when the extruded product was refrigerated at 4°C for 24 h before oven drying, the RS3 content increased slightly. Generally, the Phoenix variety exhibited a higher RS3 content than CDC-Candle. This was attributed mainly to the higher amylose content of Phoenix.

An attempt was made to isolate the RS3 from the extruded product using enzymes. The methodology involved sequential enzymatic treatment of the barley flours with lichenase, β-glucosidase, protease, thermostable α-amylase and amyloglucosidase. The RS3 content of the isolates were lower than that of the raw material (extruded flour) before enzymatic isolation. This indicated that RS3 did not concentrate in the isolate. It is likely, the hydrolysis of the other components such as β-glucans and proteins may have exposed RS1 and RS2 (that were previously embedded in the β-glucans/proteins matrix) to α-amylase.

The amount of β-glucan in the extruded product did not change significantly under the different extrusion conditions. This is ideal for the development of extruded products containing barley flour as an ingredient. However, molecular degradation of β-glucan may occur and these could affect some of the health benefits that have been attributed to β-glucan.

The potential of utilizing barley as a source of human food is immense and, this aspect is gaining momentum due to the demonstrated health benefits attributed to β-glucans. To be commercially viable, the grain material left after β-glucan extraction (fractionation) must be exploited and used in other food or non-food formulations. Starch, the major component of most cereals including barley, can be recovered during the fractionation of β-glucan. This has been achieved at the laboratory level and needs to be scaled up for commercial production

FDA's approval in 1997 of a health claim for certain types of oat products has boosted the sales of oat products and has attracted the attention of food manufacturers to barley β-glucan as it is more easily available in large quantities at a lower price. However, this interest in the utilization of barley components can be increased tremendously if the health benefits and functionality of barley β-glucan and starch can be substantiated with scientific evidence. This may eventually lead to the FDA and other regulatory agencies to approve a health claim for barley similar to that of oat. A similar claim for oatrim® has recently been allowed (FDA, 2002)

In conclusion, the *in vitro* PAA-assisted hydrolysis of gelatinized starch is influenced by the concentration and molecular weight of barley β-glucan. Extrusion cooking of barley floor does not induce RS3 formation. However, refrigeration of extruded barley flour at 4°C increased RS3 content. Post extrusion refrigeration can increase RS3 in the extruded barley flour.

Nutritional research has clearly demonstrated that excess eating of starchy foods, (especially in the developed countries) leads to a number of health problems such as obesity, diabetes (Type II), heart diseases, hyperactivity, etc. The concept of low-glycemic foods and its health benefits has been introduced recently. The glycemic index is a ranking of starch/dextrin carbohydrates based on their immediate effect on blood glucose (blood sugar) levels. Starch and dextrin (hydrolysed starch) that breakdown quickly during digestion have the highest glycemic indexes. Rapid increase in blood glucose levels leads to the aforementioned health problems. Carbohydrates that breakdown slowly, releasing glucose gradually into the blood stream, have low glycemic indexes and are beneficial to human health. Leading food industries across the world are developing new varieties of low glycemic foods in order to address the problems related to high glycemic foods as well as to capitalize on the fast growing market demand for low glycemic foods.

The research results presented in Chapters 2 and 3 of this thesis clearly indicate that β-glucan influences starch hydrolysis and therefore, can be used in the formulation of low glycemic foods. The approach to development of low glycemic foods is primarily two fold: a) reduction of the actual starch/dextrin content of foods (low-starch and low glycemic). These foods are usually enriched in protein (i.e. soy or whey protein concentrate) and b) use of chemical/physical modification of starch or dextrins that resist digestion or slowly digested in the human intestinal tract. Currently, the former approach is popular especially in the nutritional bar and beverage industry. However, developing low glycemic meal with low/no starch negatively influences the sensory quality of foods and thus the demand for the latter approach is fast growing. The preparation of low glycemic foods by incorporating a non-starch ingredient such as β-glucan to suppress starch digestibility differ from the aforementioned approaches (a and b above) and therefore, should be considered a novel food product development.

6.2 DIRECTIONS FOR FUTURE RESEARCH

1. Further research needs to be done to investigate and determine specifically what other components are responsible for the increase in viscosity during the interaction of PAA hydrolytic products and low purity β-glucan concentrate.
2. Research must be conducted to investigate how incorporation of β-glucan in food products containing protein, carbohydrates and lipids in varying proportion influences β-glucan solubility and ability to lower cholesterol and attenuate post-prandial serum glucose and insulin.
3. *In vitro* studies provided information on the effects of β-glucan /PAA hydrolytic products on starch hydrolysis. However, in order to obtain data that can be closely extrapolated to humans, an *in vivo* model should be developed to further investigate the effect of β-glucan /PAA hydrolytic products and its implications on starch hydrolysis and absorption.
4. Research is needed to investigate the implications of extrusion induced β-glucan fragmentation on the nutritional quality of barley flour in human health.
5. It is still not clear whether β-glucan solution viscosity slows amylolysis or impedes the absorption of hydrolytic products or both. An *in vitro* model can be developed to try to elucidate this discrepancy. I am suggesting that starch hydrolysis be performed in a dialysis tubing set up (Appendix VI) and the hydrolytic products monitored within and outside the tubing.

6.3 REFERENCES

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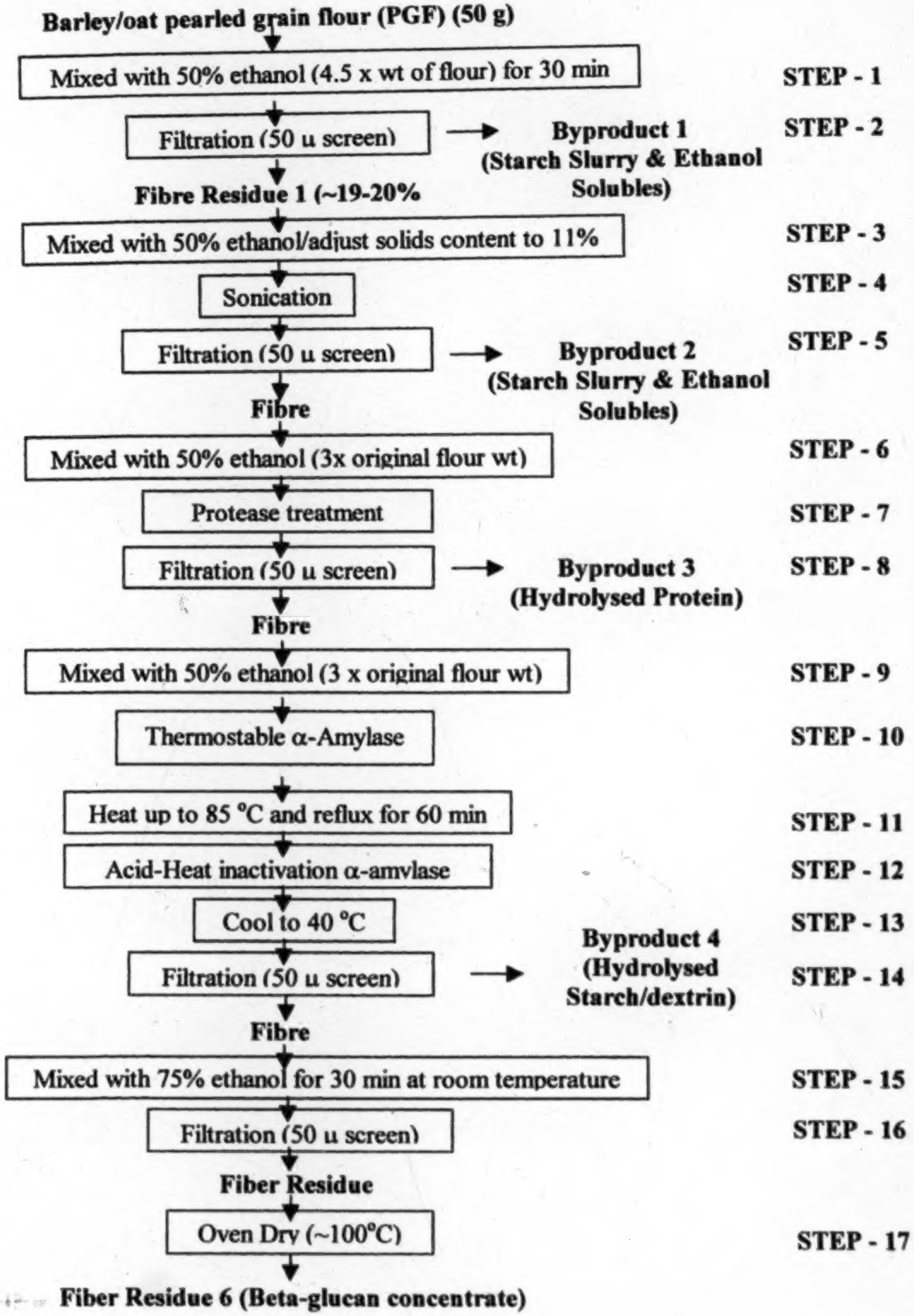
Zvonko, B. 2004. Department of Agricultural, Food and Nutritional Science, University of Alberta. Edmonton, AB. Canada. Personal communication.

Appendix 1

Laboratory β-glucan isolation process by Vasanthan and Temelli (2002)


Barley/oat pearled grain flour (PGF) (50 g)
Mixed with 50% ethanol (4.5 x wt of flour) for 30 min
STEP - 1
Filtration (50 u screen)
Byproduct 1 (Starch Slurry & Ethanol Solubles)
STEP - 2
Fibre Residue 1 (~19-20%
Mixed with 50% ethanol/adjust solids content to 11%
STEP - 3
Sonication
STEP - 4
Filtration (50 u screen)
Byproduct 2 (Starch Slurry & Ethanol Solubles)
STEP - 5
Fibre
Mixed with 50% ethanol (3x original flour wt)
STEP - 6
Protease treatment
STEP - 7
Filtration (50 u screen)
Byproduct 3 (Hydrolysed Protein)
STEP - 8
Fibre
Mixed with 50% ethanol (3 x original flour wt)
STEP - 9
Thermostable α-Amylase
STEP - 10
Heat up to 85 °C and reflux for 60 min
STEP - 11
Acid-Heat inactivation α-amylase
STEP - 12
Cool to 40 °C
STEP - 13
Filtration (50 u screen)
Byproduct 4 (Hydrolysed Starch/dextrin)
STEP - 14
Fibre
Mixed with 75% ethanol for 30 min at room temperature
STEP - 15
Filtration (50 u screen)
STEP - 16
Fiber Residue
Oven Dry (~100°C)
STEP - 17
Fiber Residue 6 (Beta-glucan concentrate)

Beta-glucan Isolation Process

The isolation process shown in Appendix 1 is simply a concentration process. The process consists of five major unit operations: a) ethanol wash (Step 1), b) sieving (Step 2), c) sonication (Step 4), protease treatment (Step 7) and d) amylase treatment (Step 10). The process is carried out in aqueous alcohol media in order to: 1) minimize microbial growth, naturally occurring enzymes (i.e. cellulase, lichenase, etc) and β-glucan degradation and 2) maximize the activity of protease and α-amylase.

Ethanol wash (STEP 1)

These steps are carried out to wash away from flour most of free starch and protein bodies and ethanol soluble components.

Screening streams (Step 2):

- Most of the fiber/cell wall components (>50 μ in size) are retained by the screen (**Fiber Residue 1**). The residue is then subjected to sonication and screening process through STEPS 3, 4 and 5.
- Most of free starch, protein and ethanol solubles go through the screen (**by-product 1a &1b**). The by-products are then centrifuged to separate solids (mainly starch) from liquid phase (mainly ethanol and solubles). The solid fraction is dried to obtain crude starch.

Ultrasonication (STEPS 3, 4 and 5): Fiber Residue 1 is treated with sonic waves (ultrasonication) to release more free starch and protein from fiber particles, which are subsequently screened similar to that in Step 2 using a 50 micron screen.

Protease treatment (STEPS 6,7 and 8): The protease* treatment on the fiber particles (fiber residue 2) is carried out in 50% ethanol for 2h at ambient temperature in order to hydrolyse and release protein bodies attached to fiber particles (that were removed by screening).

*Amount of added protease (kg) = 0.002x flour wt.

Amylase treatment (STEPS 9-14): Amylase* treatment is carried out in 50% ethanol on the fiber particles in order to hydrolyse and release starch granules that are attached to fiber particles (that were removed by screening).

*Amount of added amylase (litres) = 0.002-0.004 x original flour wt.

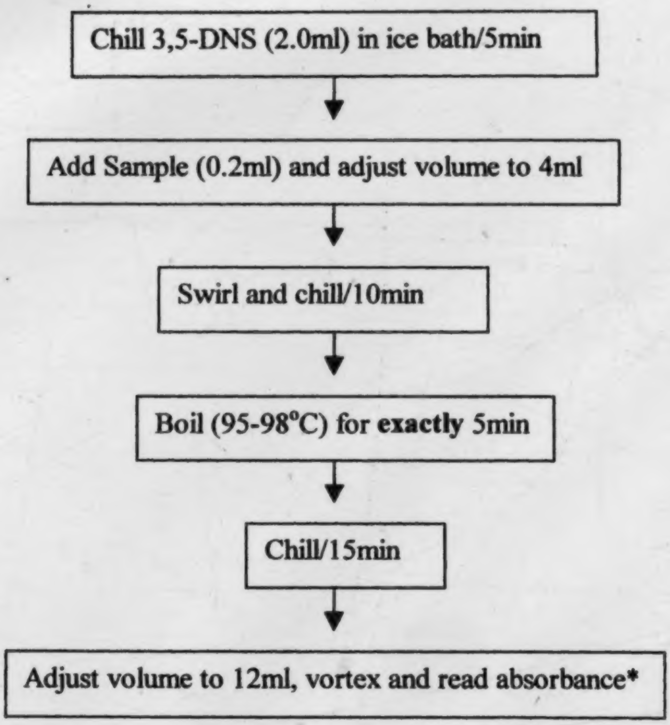
APPENDIX II

Determination of reducing value using the 3, 5-dinitrosalicylic acid procedure of Bruner (1964).

Preparation of reagents:

1. 20g of 3,5-dinitrosalicylic acid (3, 5-DNS) was dissolved in 700ml 1.0N NaOH and made up to 1L with milliQ water. The solution was then filtered through Whatman paper (No. 2). It was kept in a dark bottle covered with aluminum foil until required for analysis.
2. Maltose standard (27.6 mM, stock solution) was prepared by dissolving 500mg maltose into 50ml milliQ water.

Procedure:



Chill 3,5-DNS (2.0ml) in ice bath/5min

Add Sample (0.2ml) and adjust volume to 4ml

Swirl and chill/10min

Boil (95-98°C) for **exactly** 5min

Chill/15min

Adjust volume to 12ml, vortex and read absorbance*

* The absorbance readings were taken at 540 and 590nm for the low and high concentration curves, respectively.

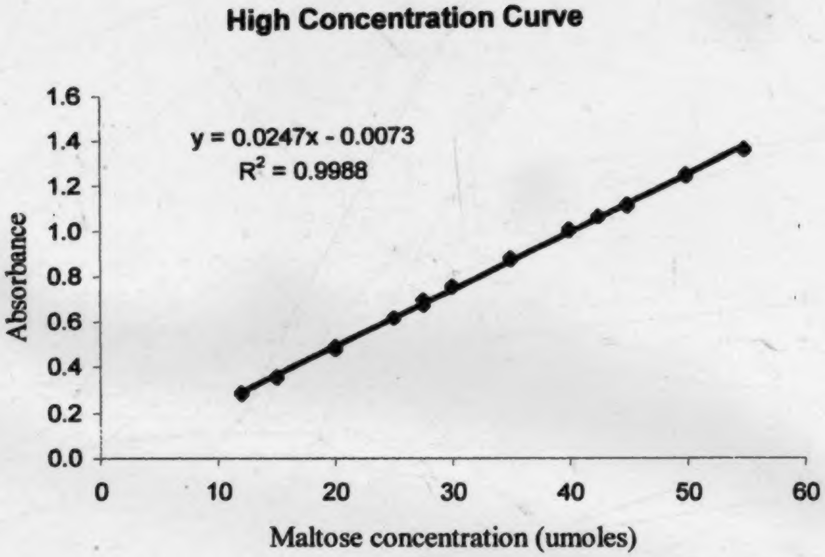
Standard curve:

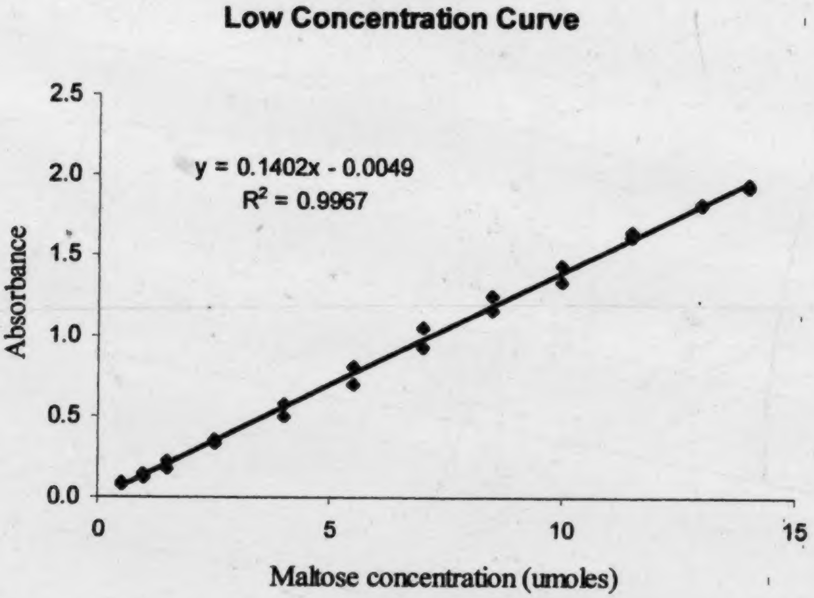
The absorbance readings are then used to create a standard curve and the linear regression line determined. Two standard curves (low and high concentration curves) were created. The standard (maltose) used for the determination of reducing sugar ranged from 0.5-14 μmoles and 12-55 μmoles.

Calculations:

The appropriate standard curve is used to determine the moles of maltose in 0.2ml aliquot. The moles of maltose are then used to calculate the total maltose formed and the degree of digestion (reducing value) using the following formulae:

- μmoles of maltose in 0.2ml aliquot (at time=t) is obtained from the standard curve regression.
- Total calculated maltose = [(μmoles of maltose (at time=t) - μmoles of maltose (at time 0) x vol of hydrolysis slurry]/0.2ml.
- Weight of glucose equivalents produced = Total calculated maltose (μmol) x mwt glucose (g/mol)/ 1000(μg/mg).
- Degree of hydrolysis (reducing value) at time t (%) = [(wt of glucose equivalents) x 100]/ wt of starch (db).


High Concentration Curve
y = 0.0247x - 0.0073
R² = 0.9988
Absorbance
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
Maltose concentration (umoles)


Low Concentration Curve
y = 0.1402x - 0.0049
R² = 0.9967
Absorbance
2.5
2.0
1.5
1.0
0.5
0.0
0
5
10
15
Maltose concentration (umoles)

APPENDIX III

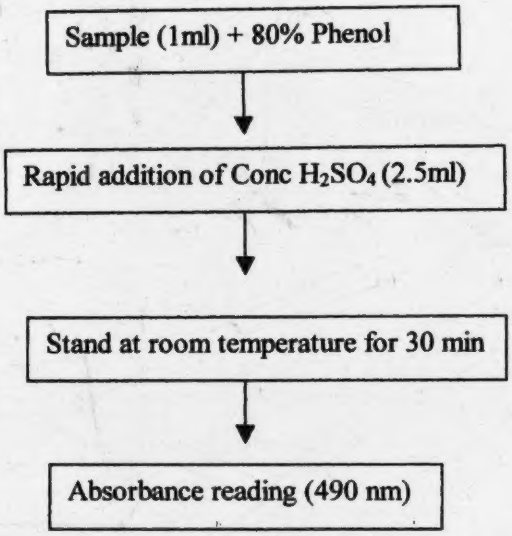
Phenol-sulfuric acid method for total sugar determination (Dubois, 1956)

Reagents:

Concentrated sulfuric acid

80% phenol solution

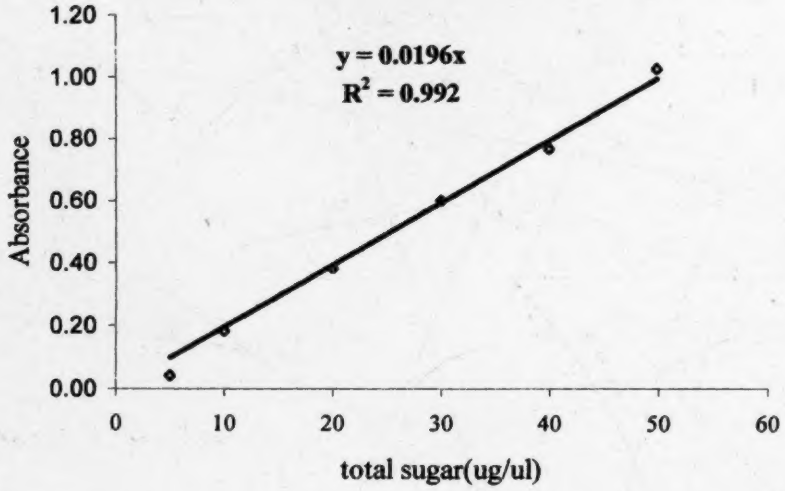
Procedure:


Sample (1ml) + 80% Phenol
Rapid addition of Conc H_2SO_4 (2.5ml)
Stand at room temperature for 30 min
Absorbance reading (490 nm)

Standard curve:

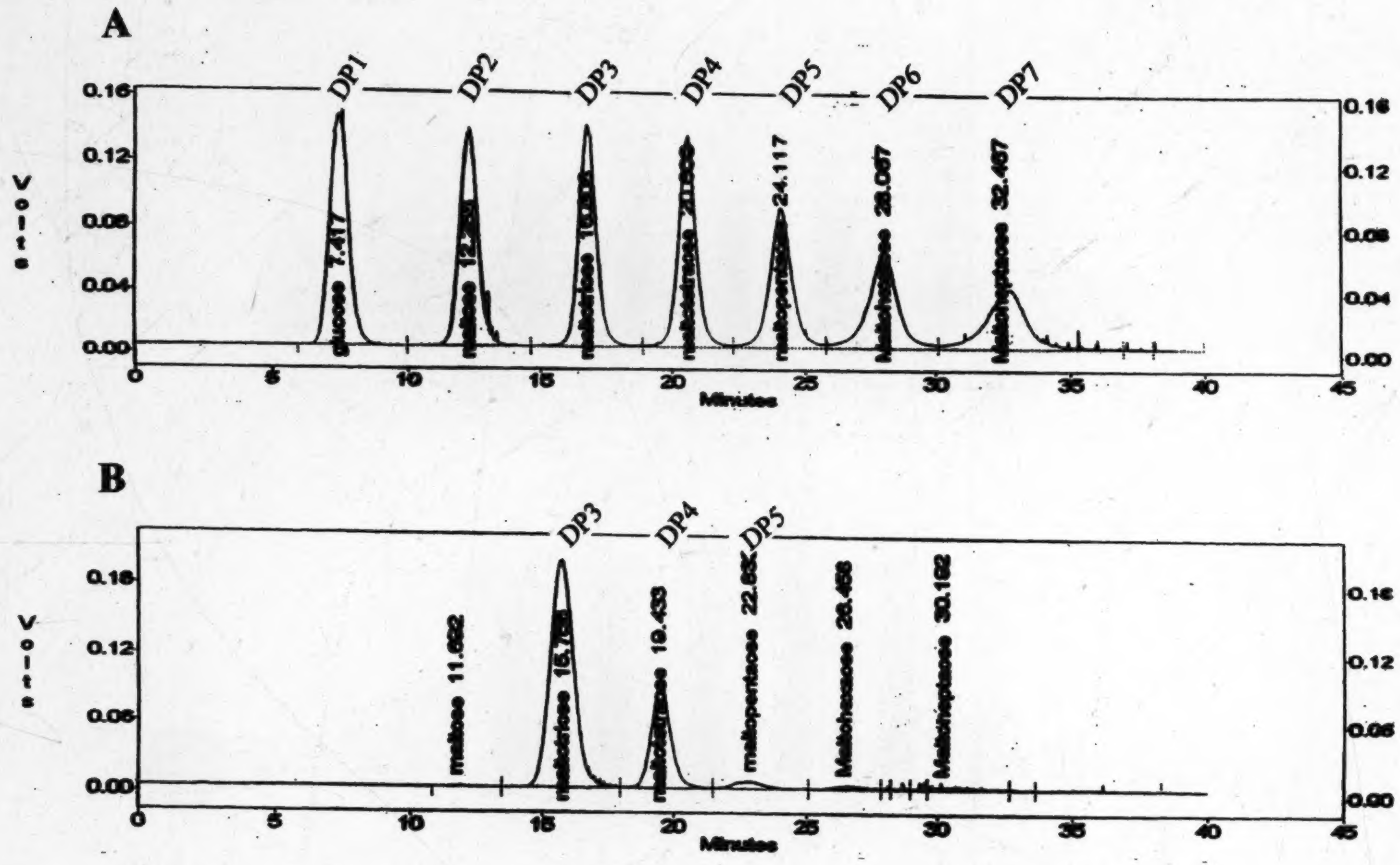
A standard curve was obtained by using appropriately diluted series of glucose standards made from a stock solution (0.1g/µl) solution. The phenol-sulfuric reaction was carried out as described above for the samples. A standard curve was generated from the absorbance readings.

Standard Curve for Total Sugar determination

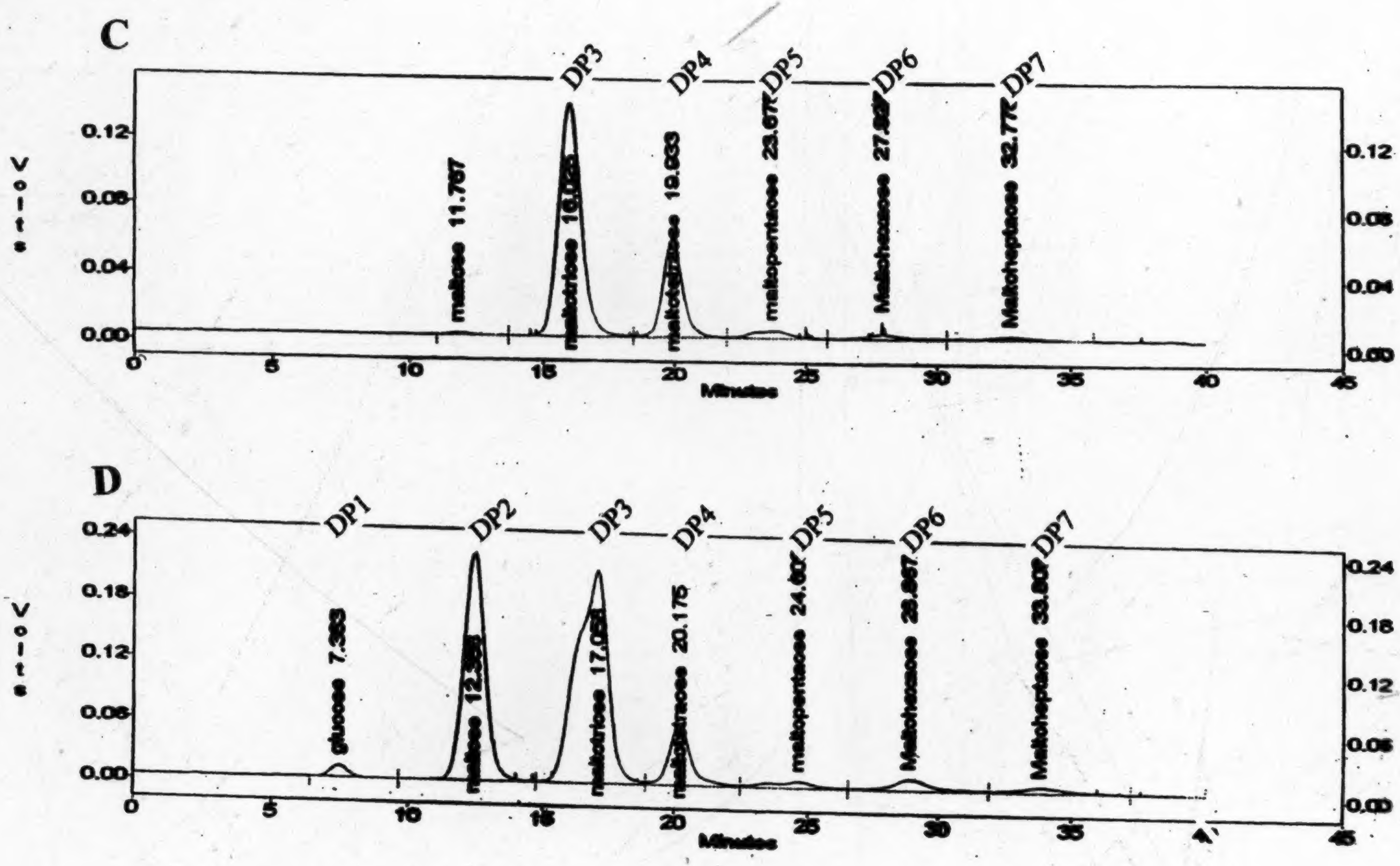


APPENDIX IV

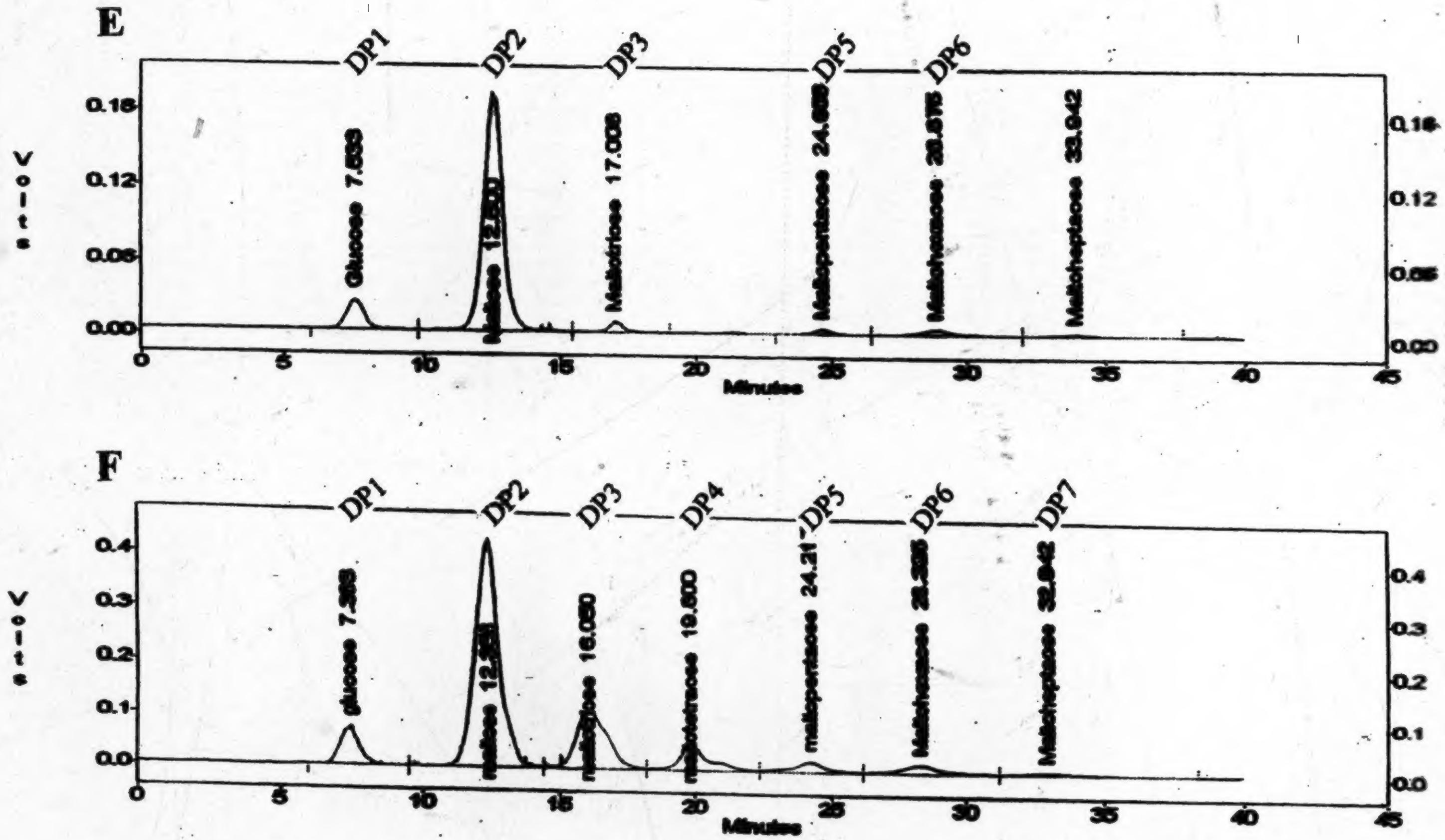
Representative HPLC chromatograms for sugar standards (A), hydrolysed β-glucan (B), hydrolysed β-glucan plus gelatinized starch and PAA at 0 (C), 1 (D) and 24 h (F), and native β-glucan plus gelatinized starch and PAA at 24h (E) of PAA hydrolysis.



A=Sugar standards and B= hydrolysed β-glucan only



Hydrolysed β-glucan plus gelatinized starch and PAA at 0 h = C and 1 h =D



Native β-glucan plus gelatinized starch and PAA at 24h = E and hydrolysed β-glucan plus gelatinized starch and PAA at 24 h = F

APPENDIX V

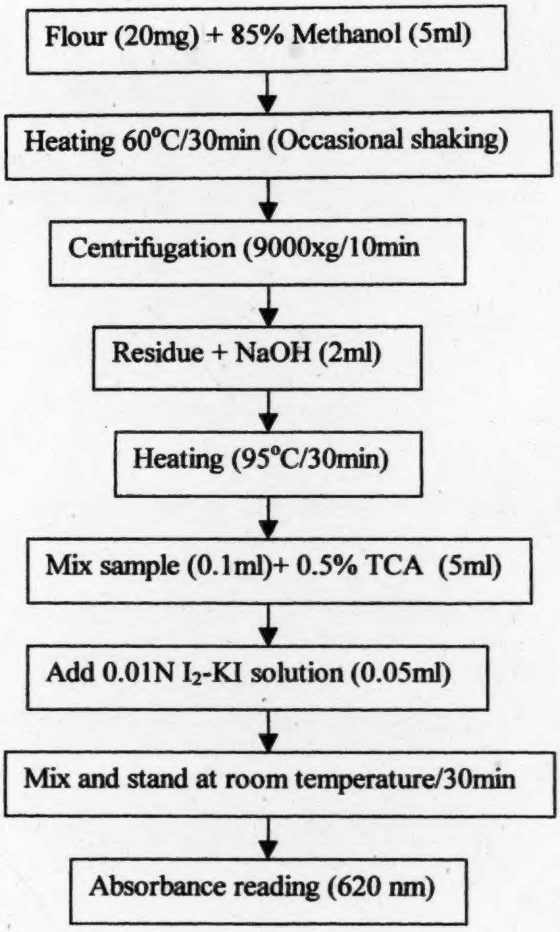
Determination of amylose (Chrastil, 1987)

Reagents:

85% Methanol, 1N NaOH, I_2 KI

0.5% Trichloroaxcetic acid (TCA)

Procedure:

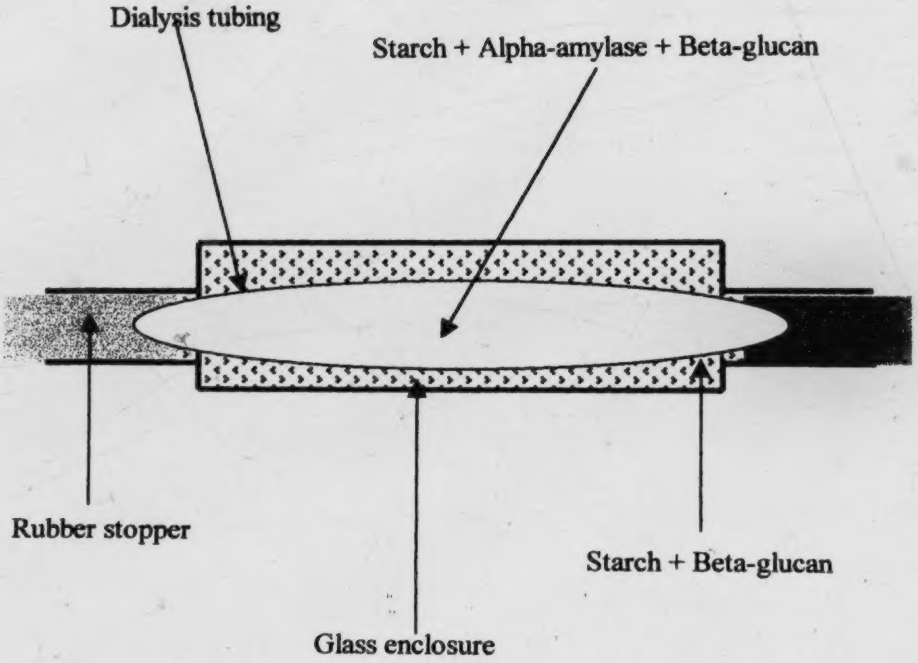

Flour (20mg) + 85% Methanol (5ml)
Heating 60°C/30min (Occasional shaking)
Centrifugation (9000xg/10min
Residue + NaOH (2ml)
Heating (95°C/30min)
Mix sample (0.1ml)+ 0.5% TCA (5ml)
Add 0.01N I₂-KI solution (0.05ml)
Mix and stand at room temperature/30min
Absorbance reading (620 nm)

Standard curve:

A standard curve was obtained from duplicate determination of amylose solution of various concentrations.

APPENDIX VI

In vitro hydrolysis using a dialysis tubing


Dialysis tubing
Starch + Alpha-amylase + Beta-glucan
Rubber stopper
Starch + Beta-glucan
Glass enclosure

An attempt can be made to perform the *in vitro* α-amylolysis of barley starch with and without β-glucan in a dialysis tubing (cut-off molecular weight of 3,500 daltons) that is previously soaked in milliQ water overnight. Starch (5g) in 200 ml buffer (pH 6.9) is gelatinized and β-glucan added at the concentrations specified in section 3.2.2.3.1. A portion (50 ml) of this starch-β-glucan slurry is then poured into a glass covered dialysis tubing (Figure above). The remaining 150 ml of the slurry is then poured into the space between the dialysis tubing and the glass enclosure. This is performed in order to balance the osmotic pressure. The set up shown in the above figure is then placed in a thermostatically controlled chamber at 37°C for 1h before the addition of α-amylase (4units/mg of dry starch). At different time periods of hydrolysis, aliquots of 5 ml and 15ml is taken from within the dialysis tubing and from the space between the dialysis tubing and the glass enclosure, respectively. The aliquots are immediately boiled in a water bath (95-98 °C for 10 min in order to inactivate α-amylase and then immediately frozen in liquid nitrogen and stored at –18 °C until required for reducing sugar determination analysis. By monitoring the reducing values inside and outside the dialysis tubing, the trend of the hydrolytic products may be determined

Publications:

Refereed Journals:

1. Faraj, A. Vasanthan, T., and Hoover, R. 2004. The effect of extrusion cooking on resistant starch formation in waxy and regular barley flour. Food Res Int. 37:517-535.
2. Faraj, A., and Vasanthan, T. 2004 Soybean isoflavones: Effects of processing and health benefits. 2004. Food Rev. Int. 20: 51-75.
3. Faraj, A., Vasanthan, T., and Hoover, R. 2004. The influence of beta-glucan concentration on the *in vitro* hydrolysis of barley starch by porcine pancreatic alpha-amylase. Cereal Chem. (under review).
4. Faraj, A., Vasanthan, T., and Hoover, R. 2004. Solution viscosity of low and high purity barley beta-glucan concentrates as influenced by dextrin fractions of alpha-amylase hydrolysed starch. Food Chem. (under review).

Proceedings and Conferences:

1. Faraj, A., and Vasanthan , T. 2003. In vitro alpha-amylase hydrolysis of barley starch as influenced by (1-3)(1-4)-D-beta-glucan concentration in an aqueous slurry. Oral presentation by A. Faraj at the Annual American Association of Cereal Chemists Meeting, Portland, Oregon, USA.
2. Faraj, A., and Vasanthan, T. 2002. Effect of extrusion cooking on the formation of resistant starch in waxy and regular barley. Poster presented by A. Faraj at Annual American Association of Cereal Chemists Meeting, Oct 13-17, 2002. Montreal Canada.